Polar & Alpine Microbiology

6th International Conference on Polar and Alpine Microbiology

September 6 – 10, 2015

Centre for Polar Ecology, Faculty of Science,
University of South Bohemia in České Budějovice
České Budějovice

Programme and Abstracts

Edited by Jana Kvíderová, Daria Tashyreva,
Alexandra Bernardová & Josef Elster

PAM 2015

Přírodovědecká fakulta
Faculty of Science

IASC

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THE INTERNATIONAL COUNCIL FOR SCIENCE
SCIENTIFIC COMMITTEE ON ANTARCTIC RESEARCH

INSTITUTE OF BOTANY ASCR

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International Arctic Science Committee
Scientific Committee for Antarctic Research
Faculty of Science, University of South Bohemia in České Budějovice (project no. IP15 PO 03)
Institute of Botany AS CR (in frame of long-term research development project No. RVO 68985939)

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Message from the Chair of the PAM2015 Conference

Dear Colleagues,

Since the 1st Polar and Alpine Microbiology Conference held in Rovaniemi the world microbial society studying polar and alpine microbes has been meeting regularly every two years (Rovaniemi, Finland 2004, Innsbruck, Austria 2006, Banff, Canada 2008, Ljubljana, Slovenia 2011, Big Sky, USA 2013 and České Budějovice, Czech Republic 2015).

It is our great privilege to host the conference in our country this year. Czech Republic (and Slovak Republic, former Czechoslovakia) have been members of the alpine and later polar science community. At the end of the last century, with opening of the borders between East and West and political changes in Central and East Europe, Czech polar activities started to flourish. Several expeditions to various parts of the Arctic and Antarctic have been organized. At present two polar research infrastructures are managed by the Czech Republic. Since then, the Svalbard archipelago and Antarctic Peninsula are the main regions of our interest. The Czech Arctic Research Station of Josef Svoboda on Svalbard is managed by the Centre for Polar Ecology, Faculty of Science, University of South Bohemia in České Budějovice while the Czech Antarctic research station of J.G. Mendel on James Ross Island is managed by the Institute of Geography, Faculty of Science, Masaryk University in Brno.

The conferences were always very successful in bringing together the scientific community for discourse on the latest in all aspects of cold-living microorganisms and their role in polar and alpine environments. Climate changes that were observed and documented over the last decades brought polar and alpine areas to the center of attention of the general public and international science community, including microbiologists. Understanding the processes occurring across polar and alpine environments requires a coordinated effort over space and time to capture the naturally high variability associated with Polar and Alpine Regions.

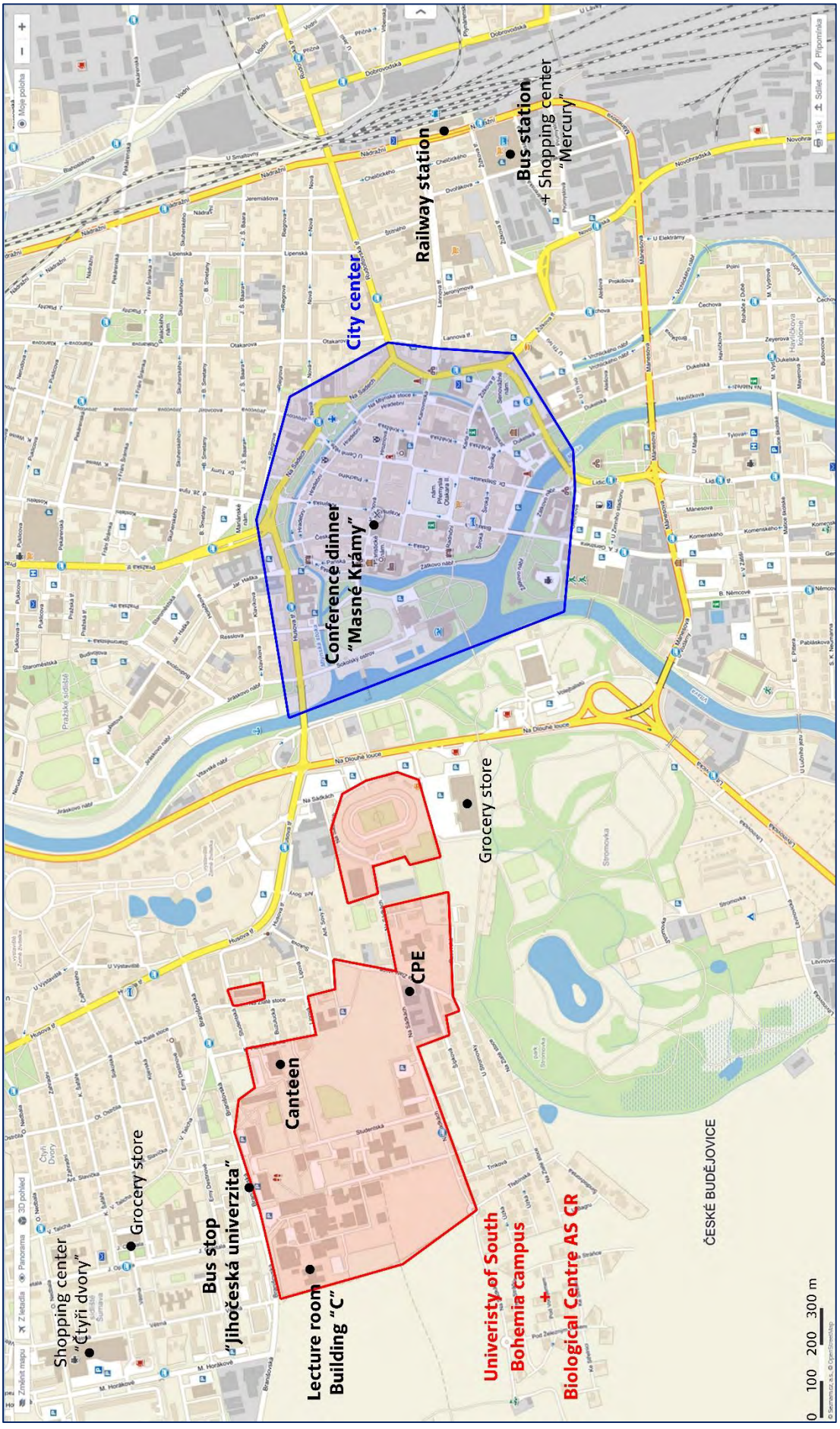
The conference is organized by the Centre for Polar Ecology and I would like to acknowledge support for this conference from the Faculty of Science, University of South Bohemia in České Budějovice, the Institute of Botany, Academy of Science of the Czech Republic, Třeboň and the international polar organizations - the International Arctic Science Committee and the Scientific Committee for Antarctic Research.

Welcome to České Budějovice, enjoy the Polar and Alpine Microbiology Conference, and enjoy your stay in the beautiful region of South Bohemia!

Josef Elster

Chair of the Conference
Head of the Centre for Polar Ecology

Map of facilities



Conference programme

Conference schedule

	Sun September 6	Mon September 7	Tue September 8	Wed September 9	Thr September 10
8:00		Registration (Building C lobby)	Registration (Building C lobby)	Registration (Building C lobby)	
8:20		Welcome speech (Lecture room)			
8:30		Polar/alpine microbiology and environmenral change (Lecture room)	Microbial diversity and evolution (Lecture room)	Supraglacial, subglacial and glacial microbiology (Lecture room)	Exursion (South Bohemia)
10:00		Coffee break (Building C lobby)	Coffee break (Building C lobby)	Coffee break (Building C lobby)	
10:30		Polar/alpine microbiology and environmenral change (Lecture room)	Microbial diversity and evolution (Lecture room)	Supraglacial, subglacial and glacial microbiology (Lecture room)	
11:50				Lunch (Canteen)	
12:10		Lunch (Canteen)	Lunch (Canteen)		
13:00		Cold physiology and cryobiology (Lecture room)	Microbial diversity and evolution (Lecture room)	Supraglacial, subglacial and glacial microbiology (Lecture room)	
14:00			Coffee break (Building C lobby)	Coffee break (Building C lobby)	
14:30		Coffee break (Building C lobby)	Polar/alpine eukaryotic microorganisms (Lecture room)	Astrobiology of icy worlds (Lecture room)	
15:00		Cold physiology and cryobiology (Lecture room)			
16:00	Registration (Building C lobby)		Coffee break (Building C lobby)	Coffee break (Building C lobby)	
16:20		Coffee break (Building C lobby)			
16:30		Poster Session A Official part (Building C lobby)	Polar/alpine cyanobacteria (Lecture room)	Biotechnology at low temperatures (Lecture room)	
17:20			Coffee break (Building C lobby)		
17:30		Poster session A with Budvar beer (Building C lobby)	Poster Session A Official part (Building C lobby)		
18:00	Opening ceremony (Building C lobby)			Closing ceremony (Lecture room)	
18:30	Icebreaker party (Building C lobby)		Poster session B with Budvar beer (Building C lobby)		
19:00					
19:10					
19:50					
20:00				Conference Dinner (Masné krámy)	
22:00					

Conference programme

Sunday September 6, 2015

16:00	-	21:00	**Registration**
18:00	-	18:30	**Opening ceremony**
18:30	-	22:00	**Icebreaker party**

Monday September 7, 2015

8:00	-	17:00	**Registration**
8:20	-	8:30	**Welcome speech**

A. Polar/alpine microbiology and environmental change: past, present and future

Minna K. Männistö (Chair)
Finnish Forest Research Institute, Rovaniemi, Finland
Max Häggblom (Co-Chair)
Rutgers University, USA

8:30	-	9:00	Minna K. Männistö	The impact of large grazers on the responses of soil microbial communities to warming and increased nitrogen avaiability	KN-A
9:00	-	9:20	Alexandre Anesio	Microbial succession from ice to vegetated soils in the High Arctic	L-A-01
9:20	-	9:40	Craig Cary	Resolving spatial and temporal heterogeneity in terrestrial Antarctic microbial communities	L-A-02
9:40	-	10:00	Max Häggblom	Bacterial utilization of carbon and nitrogen at subzero temperatures in tundra soils	L-A-03
10:00	**-**	**10:30**	**Coffee break**		
10:30	-	10:50	Elisabeth Helmke	Arctic bacterial sea ice communities affected by global warming	L-A-04
10:50	-	11:10	Anne Jungblut	Microbial mat communities along environmental gradients in perennially ice covered Antarctic lakes	L-A-05
11:10	-	11:30	Gabriela Mataloni	Microbial planktonic communities as environmental indicators in a Tierra del Fueglo peat bog	L-A-06
11:30	-	11:50	Laura Selbmann	Environmental pressure and variation of fungal biodiversity in rock microbial communities of Northern Victoria Land (Antarctica)	L-A-07
11:50	-	12:10	Ruben Sommaruga	Changes in bacterial community composition along a turbidity gradient in recently-formed lakes in SW Greenland	L-A-08
12:10	**-**	**13:00**	**Lunch**		

C. Cold physiology and cryobiology

Anders Priemé (Chair)
Geological Survey of Denmark and Greenland, Copenhagen, Denmark
Antonio Quesada (Co-Chair)
Universidad Autónoma de Madrid, Spain

13:00	-	13:30	Andres Priemé	Microbial activity in newly thawed permafrost soil	KN-C
13:30	-	13:50	Klaus Herburger	Callose acts against desiccation: induced forces in filamentous streptophyte green algae from alpine regions	L-C-01
13:50	-	14:10	Thulani Makhalanyane	Meta-omic analysis reveals widespread functionality in Antarctic hypoliths from two Dry Valley system	L-C-02
14:10	-	14:30	Riitta Nissinen	Some like it cold, some like it green, some like it cold and green - comparative genomics of sphingomonads associaated with Arctic plants	L-C-03
14:30	**-**	**15:00**	**Coffee break**		
15:00	-	15:20	Elena Patova	Nitrogenase activty of soil cyanobacterial crusts in polar and subpolar Urals (European North-East Russia)	L-C-04
15:20	-	15:40	James Raymond	Ice binding proteins of a snow alga, *Chloromonas brevispina*: probable acquisition by horizontal gene transfer	L-C-05
15:40	-	16:00	Daria Tashyreva	The limits of desiccation tolerance of Arctic *Microcoleus* strains (Cyanobacteria) and environmental factors inducing it	L-C-06
16:00	-	16:20	Cinzia Verde	Coping with cold: from the structure to the function of Antarctic bacterial globins	L-C-07
16:20	**-**	**16:30**	**Coffee break**		

Poster session A

16:30 - 17:30 Official part - Posters from Sections A, C, D and G; followed by beer party

Tuesday September 8, 2015

8:00 - 17:00 **Registration**

B. Microbial diversity and evolution

David Pearce (Chair)
Northumbria University, Newcastle, UK
Dirk Wagner (Co-Chair)
German Research Center for Geosciences, Potsdam, Germany

8:30	-	9:00	David Pearce	So what is in the atmosphere - the last piece of the jigsaw?	KN-B

9:00	-	9:20	Natalia Belkova	Variety and diversity of representatives of 'candidate' phyla in cold seeps from Sayan Mountains (Siberia, Russia)	L-B-01
9:20	-	9:40	Martin Hartmann	Unraveling the unknown microbial diversity hidden in alpine permafrost	L-B-02
9:40	-	10:00	Christoph Keuschnig	Arctic snowpack-soil interface - strict boundary or ecosystem trading zone?	L-B-03
10:00	**-**	**10:30**	**Coffee break**		
10:30	-	10:50	Yung Mi Lee	Draft genome of members of the OP9 lineage obtained from single cells sorted from a marine sediment of the Ross Sea, Antarctica	L-B-04
10:50	-	11:10	John Priscu	Methane transormations in Arctic and Antarctic ice-covered lakes	L-B-05
11:10	-	11:30	Sara Rassner	It pays to be a winner: viral control of the bacterial community of a High Arctic glacier surface	L-B-06
11:30	-	11:30	Elizaveta Rivkina	Metagenomics of permafrost - key for paleoecology	L-B-07
11:50	-	12:10	Viktoria Shcherbakova	Sulfate-reducing bacteria in Arctic gryopegs	L-B-08
12:10	**-**	**13:00**	**Lunch**		
13:00	-	13:20	Guillaume Tahon	Diversity of *cbbL*, *nifH* and *pufLM* genes in soils around the Princess Elizabeth Station, Sør Rondane Mountains, Antarctica	L-B-09
13:20	-	13:40	Bernhard Tschitschko	Host-virus interaction in a frigid, hypersaline Antarctic lake revealed by metaproteomics	L-B-10
13:40	-	14:00	Marc Van Goethem	Microbial communities of Antarctic soil and lithic habitats	L-B-11
14:00	**-**	**14:30**	**Coffee break**		

F. Polar/alpine eukaryotic microorganisms

Wim Vyverman (Chair)
Ghent University, Belgium
Nina Gunde-Cimerman (Co-Chair)
University of Ljubljana, Slovenia

14:30	-	15:00	Wim Vyverman Elie Verleyen	Post-Miocene divergence of polar diatom biomes	KN-F
15:00	-	15:20	Nina Gunde-Cimernan	Black yeasts from glaciers to sauna - biological answer to a changing world?	L-F-01
15:20	-	15:40	Maxime Sweetlove	Biogeographic zoning of aquatic microeukaryotes in the Antarctic realm	L-F-02
15:40	-	16:00	Tatiana Vishnivetskaya	Hunting for green algae and cyanobacteria in Siberian permafrost	L-F-03
16:00	**-**	**16:20**	**Coffee break**		

E. Polar/alpine cyanobacteria

Jiří Komárek (Chair)
Academy of Science, Institute of Botany, Třeboň, Czech Republic
Annick Wilmotte (Co-Chair)
Liege University, Belgium

16:30	-	17:00	Jiří Komárek	Polar/Alpine cyanobacteria	KN-E
17:00	-	17:20	Antje Donner	Diversity of hypolithic cyanobacteria from three locations in western Spitsbergen	L-E-01

17:20 - 17:30 Coffee break

Poster session B

17:30 - 18:30 Official part - Posters from Sessions B, F, E and H; followed by beer party

Wednesday September 9, 2015

8:00 - 12:00 **Registration**

D. Supraglacial, subglacial and glacial microbiology

Andy J. Hodson (Chair)
University of Sheffield, UK
Marek Stibal (Co-Chair)
Charles University, Prague, Czech Republic

8:30	-	9:00	Andy J. Hodson	The ecology and biogeochemistry of maritime Antarctic snow	KN-D
9:00	-	9:20	Liz Bagshaw	Light adaptation of microbial communities in Antarctic cryoconite holes	L-D-01
9:20	-	9:40	Karen Cameron	Export of microbial cells from the Greenland Ice Sheet	L-D-02
9:40	-	10:00	Andrea Franzetti	Dynamics and microbial community functions in cryoconite from Italian Alps and Karakoram	L-D-03

10:00 - 10:30 Coffee break

10:30	-	10:50	Stefanie Lutz	Biogeography and functionality of microbial glacial surface communities across the Arctic	L-D-04
10:50	-	11:10	Lorrie Maccario	Microbial life in the arctic snowpack photochemical reactor	L-D-05
11:10	-	11:30	Birgit Sattler	Settlement of an Alpine englacial system with microbial communities - who comes first?	L-D-06
11:30	-	11:50	Takahiro Segawa	The nitrogen cycle in cryoconites: naturally occurring nitrification-denitrification granules on a glacier	L-D-07

11:50 - 13:00 Lunch

13:00	-	13:20	Mark Skidmore	Linking elemental cycles in subglacial systems through microbial processes	L-D-08

13:20	-	13:40	Marek Stibal	The role of ice algae in the albedo feedback on the Greenland Ice Sheet	L-D-09
13:40	-	14:00	Jon Telling	Between a rock and a hard place: rock comminution as a source of hydrogen for subglacial systems	L-D-10
14:00	**-**	**14:30**	**Coffee break**		

H. Astrobiology of icy worlds

Jean-Pierre Paul de Vera (Chair)
Institute of Planetary Research, Berlin, Germany
Silvano Onofri (Co-Chair)
Università della Tuscia, Italy

14:30	-	15:00	Jean-Pierre de Vera	Potential biospheres in the icy worlds in our solar system	KN-H
15:00	-	15:20	Sergey Bulat	Microbiology of the subglacial lake Vostok: First results with borehole-frozen lake water and prospects	L-H-01
15:20	-	15:40	Silvano Onofri	BIOMEX experiment: survival, urltrastructural and molecular damage in the cryptoendolithic Antarctic fungus *Cryomyces antarcticus* exposed to space and simulated Mars-like conditions	L-H-02
15:40	-	16:00	Dirk Wagner	*Methanosarcina soligelidi* SMA-21 - an archaeal candidate for life on Mars	L-H-03
16:00	**-**	**16:30**	**Coffee break**		

G. Biotechnology at low temperatures

Rosa Margesin (Chair)
Innsbruck University, Austria
Giuseppe Torzillo (Co-Chair)
CNR - Istituto per lo Studio degli Ecosistemi, Sesto Fiorentino, Italy

16:30	-	17:00	Rosa Margesin	Biotechmological significance of microorganisms in low temperature environments	KN-G
17:00	-	17:20	Lorena Monserrate Maggi	Bioprospecting of Hg processing micro-organisms from South Shetlands Island, Antarctica	L-G-01
17:20	-	17:40	Giuseppe Torzillo	Development of photobioreactors for low-temperature environment	L-G-02
17:40	-	18:00	Oddur Vilhelmsson	Naphatlene-degrading bacteria associated with terricolous lichens in Iceland	L-G-03
	-				
18:00	-	18:30	**Closing ceremony**		
20:00	-	22:00	**Dinner at Masne Kramy**		

Thursday September 10, 2015

	-		
8:30	-	22:00	**Excursion**

Poster session A

Poster No	Presenting author		Title	
1	Ingeborg	**Bussmann**	Methane oxidation and methane distribution around the Lena Delta, Siberia, Russia	P-A-01
2	Alica	**Chroňáková**	Microbial community development on deglaciated soils in High Arctic (Svalbard) in comparison to sub-Arctic continental regions	P-A-02
3	Miloslav	**Devetter**	Terrestrial invertebrates along a gradient of deglaciation in Svalbard: relation to microbial communities	P-A-03
4	Kateřina	**Diáková**	Microbial biomass as an indicator of carbon losses from subarctic tundra soils in changing environment	P-A-04
5	Roman	**Dial**	Snow algae increases snowmelt: results of manipulative experiment on the Harding Icefield, Alaska	P-A-05
6	Richard	**Hill**	Spatial and temporal inluences on Arctic soil microbial comminity structure	P-A-06
7	Katrin	**Hofmann**	Spatial patterns of methane-cycling microorganisms in soils of a high-alpine altitudinal gradient	P-A-07
8	Weidong	**Kong**	Diversity and succession of autotrophic microbial communities in high-elevation soils along deglaciation chronosequence	P-A-08
9	Richard	**Lamprecht**	Soil mineralization sensitivity to temperature and O_2 availability in deep peat profiles including permafrost interface	P-A-09
10	Yongqin	**Liu**	Ice cores from the Tibetian Plateau reveal microbial activity convergence related to climate and anthropogenic activity	P-A-10
11	Alena	**Lukešová**	Role of soil algae and cyanobacteria in colonization and succession on deglaciated soils in High Arctic (Svalbard) and alpine/subalpine regions (Scandinavia)	P-A-11
12	Rosa	**Margesin**	Effect of altitude and season on microbial functionalizy, community structure and abundance in alpine forest soils	P-A-12
13	Alejandro	**Mateos-Rivera**	Shifts in microbial community structure in a glacier forefield (Styggedalsbreen, Central Norway)	P-A-13
14	Luis	**Morgado**	Compositional shifts in ectomycorrhizal fungal community in response to long-term snow depth increase	P-A-14
15	Hyun-Ju	**Noh**	Complex and varying lichen microbiomes according to vertical position of thallin in *Cladonia gracilis* from King George Island, Antarctica	P-A-15
16	Krzysztof	**Romaniuk**	Impact of human presence and activity on ecology and adaptation of an Antarctic psychrophilic bacteria communities	P-A-16
17	Carolina	**Voigt**	Climate feedback of arctic ecosystems: Warming enhances nutrient turnover and alters carbon and nitrogen flux dynamics in subarctic tundra	P-A-17
18	Jana	**Voříšková**	Microbial community responses to future climate change abd seasonal variation in Arctic tundra soil	P-A-18
19	Maya	**Bar Dolev**	An antarctic sea ice bacterium that uses an Ice Binding Protein to adhere to ice	P-C-01
20	Miloš	**Barták**	Resistance of Antarctic *Nostoc* sp. colonies to dehydration assessed by chlorophyll fluorescence parameters and spectral reflectance	P-C-02

21	Peter	**Convey**	Do *Chlorella* strains respond differently to temperature stress across a global gradient?	P-C-03
22	Fariha	**Hasan**	Isolation and some unique physiological characteristics of psychrotropic fungi from Passu Glacier, Pakistan	P-C-04
23	Tyler	**Kohler**	Biotic and abiotic controls of the elemental and isotopic composition of microbial communities in McMurdo Dry Valley streams, Antarctica	P-C-05
24	Anton	**Kurakov**	Charaterization of plasmids and plasmid-encoded resistance genes found in permafrost *Acinetobacter iwoffii* strains	P-C-06
25	Jana	**Kvíderová**	Growth requirements of *Stichococcus* sp. strains isolated from Rhodope Mountains, Bulgaria	P-C-07
26	Yan	**Liao**	Proteomics and genetics of Haloarchaea from deep lake, Antarctica	P-C-08
27	Phaik-Eem	**Lim**	Photosynthesis and genomic responses of *Chlorella* species from different geographical regions to artificial ultraviolet radiation (UVR) stress	P-C-09
28	Oliver	**Müller**	Changes in structure, activity and metabolic processes of microorganisms in thawing permafrost soils from Svalbard	P-C-10
29	Felipe	**Nóbrega**	Prospection and desiccation tolerance of polar microorganisms	P-C-11
30	Ksenia	**Novototskaya-Vlasova**	The molecular basis of thermostability of coldactive esterase from psychrotrophic bacterium *Psychrobacter cryohalolentis* K5T	P-C-12
31	Amedea	**Perfumo**	A single cell view of the growth of anaerobic bacterium *Clostridium psychrophilum* at subzero temperatures	P-C-13
32	Lada	**Petrovskaya**	New autotransporter from *Psychrobacter cryohalolentis* $K5^T$: characterization and construction of cell surface display system	P-C-14
33	Martina	**Pichrtová**	Desiccation stress and resistance in polar green algae of the genus *Zygnema*	P-C-15
34	Lenka	**Procházková**	Light and temperature dependence of photosynthesis in *Chlamydomonads* isolated from snow	P-C-16
35	Daniel	**Remias**	Significant cytological and physiological differences between two green algae causing red snow in the Alps	P-C-17
36	Carina	**Rofner**	Differential utilization patterns of dissolved organic phosphorus compounds by heterotrophic planktonic bacteria	P-C-18
37	Krzysztof	**Romaniuk**	Adaptive features encoded within plasmids of arctic and antarctic *Psychrobacter* spp.	P-C-19
38	Roberta	**Russo**	Structural nad functional analysis of water-borne signaling protein pheromones from bipolar protisi ciliate, *Euplodes petzi*	P-C-20
39	Laura	**Sanguino**	Viral-host interactions in glacial ice and their adaptive significance	P-C-21
40	Iris	**Schaub**	Effect of prolonged darkness and temperature on the lipid metabolism in the benthic diatom *Navicula perminuta* fffrom the Arctic	P-C-22
41	Morten	**Schostag**	Microbial transcriptomic response to thawing and freezing of active layer permafrost soil	P-C-23
42	Purnima	**Singh**	Antifreeze protein activity in glacier cryoconites	P-C-24
43	Kateřina	**Snopková**	Cold-active antimicrobial agents produced by Antarctic pseudomonads	P-C-25

44	.	**Taha**	Phylogenetic, structural and nucleic acid binding properties of a novel type of RNA-binding (TRAM) protein from an Antarctic archaeon.	P-C-26
45	Susana	**Vazquez**	Crystal structure and expression of a putatibe phage-like protein coded in the genome of a marine Antarctic bacteria	P-C-27
46	James	**Bradley**	Microbial community dynamics in the forefield of glaciers – a modelling perspective	P-D-01
47	Beat	**Frey**	Microbial diversity of the cryosphere of the Damma glacier	P-D-02
48	Jan	**Gawor**	Arctic and Antarctic supraglacial bacterial diversity revealed by next generation metagenomics	P-D-03
49	Jarishma	**Gokul**	The biogeography of cryoconite bacterial communities on a High Arctic Ice Cap	P-D-04
50	Dorota	**Górniak**	Bacterial community composition in various supraglacial habitats of Ecology Glacier (King George Island, Antarctica)	P-D-05
51	Jakub	**Grzesiak**	Microbial community changes along the Ecology Glacier ablation zone (King George Island, Antarctica)	P-D-06
52	Takumi	**Murakami**	Survey of the glacier invertebrates and their gut microbiota	P-D-07
53	Sabrina	**Obwegeser**	Cover up – coverage of glacial surfaces with industrial fleece to reduce ablation: economic blessing or ecological spell? A symbiosis of society and science	P-D-08
54	Marie	**Šabacká**	The ecology and biogeochemistry of maritime Antarctica snow	P-D-09
55	Shiv Mohan	**Singh**	Bacterial diversity and bio-potentials of Himalayan cryoconites, and its comparison with Arctic	P-D-10
56	Jun	**Uetake**	Bacterial diversity in tropical glacier and glacier foreland in Uganda	P-D-11
57	Alejandra	**Urra**	Investigation of the proglacial zone as a modulator for nutrient fluxes in ice sheet runoff	P-D-12
58	Jakub	**Žarský**	Greenland Ice Sheet as a model for microbial macroecology and evolution	P-D-13
59	Ioan	**Ardelean**	Biosynthesis of gold nanoparticles by a cryotolerant cyanobacterium isolated from Scarisoara Ice Cave (Romania)	P-G-01
60	Heida	**Fridjonsdottir**	Bioprospecting psychrotrophic sphingomonads for hydrocarbon degradation	P-G-02
61	Maria	**Papale**	Polychlorinated biphenyl degrading bacteria from the Kongfjorden (Svalbard Islands, Norway)	P-G-03
62	Maria	**Papale**	Tolerance to heavy metals and polychlorinated biphenyl biodegradation potential by Arctic bacteria from continental Norway	P-G-04
63	Jeffrey	**Vargas-Perez**	Bioprospecting of antarctic microorganisms and their extremophiles enzymes applied in food industry (amylase)	P-G-05

Poster session B

Poster No	Presenting author		Title	
1	Antonio	**Alcamí**	Biodiversity and distribution of polar freshwater viruses	P-B-01
2	Antonio	**Alcamí**	Ecological connectivity shapes viral assemblages and variability in Antarctic environments	P-B-02
3	Corien	**Bakermans**	Attempted isolation of Acidobacteria from Antarctic permafrost	P-B-03
4	Chris	**Bellas**	Virus genomes from glacial environments reveal novel virus grpups with unusual host interactions	P-B-04
5	Amanda	**Bendia**	Microbial communities from geothermal sites of a polar active volcano (Deception Island, Antarctica)	P-B-05
6	Nadine	**Borchhardt**	Biological soil crust algae in the polar regions – biodiversity, genetic diversity and ecosystem resilience under global change scenarios	P-B-06
7	Heather	**Buelow**	Differential abundance and expression of Antarctic soil microbial communities: a metatranscriptomic analysis of taxonomic and functional diversity	P-B-07
8	Kelly	**Chan-Yam**	Characterization of microbial communities in water tracks in an Antarctic Dry Valley	P-B-08
9	Laurie	**Connell**	Fungal diversity in permanently ice covered Lake Fryxell, Antarctica	P-B-09
10	Antonella	**Conte**	Cultivable heterotrophic bacteria from Antarctic permafrost	P-B-10
11	Antonella	**Conte**	Experimental approach to the screening of prokaryotic assemblage in Antarctic permafrost	P-B-11
12	Silvia	**Coria**	Induction of multiple prophages from an Antarctic marine bacterium	P-B-12
13	Olga	**Dagurova**	Controlling factors on bacterial diversity and activity of the coastal water of Lake Baikal, Siberia	P-B-13
14	Ekaterina	**Dambinova**	Psychrophilic and psychroactive bacteria in cold springs of Northern Pribaikalie	P-B-14
15	Ekaterina	**Durdenko**	Halophilic aerobic microorganims from Alyaska cryopeg	P-B-15
16	Diego	**Franco**	Microbial diversity and community composition across depth gradient in marine sediments from Admiralty Bay, King George Island and Bransfield Strait, Antarctica	P-B-16
17	Chung Yeon	**Hwang**	Marine RNA virus communities of oceanic seawaters in the vicinity of the Antarctic Peninsula	P-B-17
18	Nataliia	**Iakovenko**	How unique are the micrometazoa of antarctic soils? The example of bdelloid rotifers	P-B-18
19	Corina	**Itcus**	Diversity of cultured ice cave microcosm	P-B-19
20	Ok-Sun	**Kim**	Comprehensive analysis of soil bacterial community structure in King George Island, Maritime Antarctica	P-B-20
21	Andrea	**Kiss**	Epi- and endophytic microbial communities of Arctic and subarctic peatland mosses	P-B-21
22	Kirill	**Krivushin**	Metagenomic insights into Antractic Dry Vallys permafrost	P-B-22
23	Emanuele	**Kuhn**	Transportation nad persistence of microbial cells in central West Antarctica	P-B-23
24	Manoj	**Kumar**	Biogeographical diversity of root associated microbial communities in arctic and alpine tundra areas	P-B-24
25	Hong Kum	**Lee**	Polar and Alpine Microbial Collection (PAMC): a culture collection dedicated to polar and alpine microorganisms	P-B-25

26	Inae	**Lee**	Genomic and phenotypic characterization of a thermophilic *Bacillus* sp. 9F isolated from deep-sea hydrothermal vent plume, Southern Ocean	P-B-26
27	Yung Mi	**Lee**	Stratification of microbial community in marine sediments of the Ross Sea, Antarctica	P-B-27
28	Li	**Liao**	Bio-mining the actinobacterial treasures of the Arctic Ocean: diversity and genetic resources	P-B-28
29	Susanne	**Liebner**	Microbial abundance and methanotrophy in degrading subsea permafrost from the Laptev Sea shelf, Siberia	P-B-29
30	Evgeniya	**Matyugina**	Diversity and ecology of microorganisms from cold seeps of National park "Alkhanai" (Transbaikalia, Russia)	P-B-30
31	Viktoriya	**Oshurkova**	The search of methanogenic archaea in Arctic and Antarctic permafrost	P-B-31
32	Cristina	**Purcarea**	Prokaryotic community structute across the ice block of Scarisoara Cave determined by 454 pyrosequencing	P-B-32
33	Ana Judith	**Russi Colmenares**	Community composition, diversity and activity of N fixing cyanobacteria associated with mosses in sub-Arctic alpine ecosystems	P-B-33
34	Iara	**Santiago**	Lichensphere: a protected natural microhabitat of the non-lichenized fungal communitieds living in extreme environments of Antarctica	P-B-34
35	Tina	**Šantl-Temkiv**	Aerial transport of bacterial cells in the Arctic: sources, deposition and impact on atmospheric processes	P-B-35
36	Bjorn	**Tytgat**	Bedrock and biotic influence on community composition in soils from the Sør Rondane Mountains, East Antarctica	P-B-36
37	Bjorn	**Tytgat**	Biogeographic patterns in Antarctic lacustrine prokaryotes	P-B-37
38	Annick	**Wilmotte**	A plea for the creation for inviolate areas to protect reference arear for future microbiology research in Antarctica	P-B-38
39	Tina	**Wunderlin**	Bacterial geography of high altitude snow -compering diversity in snow from Jungfraujoch (Switzerland) and Snowy Mountains (Australia)	P-B-39
40	Soo Jeong	**Yoon**	High occurence of thermophilic bacteria isolated from hydrothermal vent plumes in Australian-Antarctic Ridge, Southern Ocean	P-B-40
41	Marek	**Zdanowski**	Molecular characterization of miniature plasmids of Arctic psychrophilic bacteria of the genus *Variovorax*	P-B-41
42	Yinxin	**Zeng**	Diversity of *pufM* and *g5* genes in Bacterioplankton communities in coastal seawaters of Fildes Peninsula, King George Island, Antarctica	P-B-42
43	Nathan	**Chrismas**	The evolution of cold tolerance in cyanobacteria	P-E-01
44	Ľubomír	**Kováčik**	Characterization of ten strains of filamentous cyanobacteria from the South Shetland Islands , Maritime Antarctica	P-E-02
45	Anna	**Patova**	Morphological and molecular characteristics of *Nostoc commune* Vauch. ex Born. & Flah. populations in mountain and Arctic habitats	P-E-03
46	Cristina	**Purcarea**	Diversity pf phototrophic bacteria in Scarisoara Ice Cave	P-E-04
47	Ekaterina	**Pushkareva**	Impact of environmental factors on soil crust community in Svalbard	P-E-05
48	Lenka	**Raabová**	Thin and ubiquitous: taxonomic features of *Leptolyngbya* in Polar Regions	P-E-06
49	Annick	**Wilmotte**	A next-generation protocol for the assessment of cyanobacterial diversity	P-E-07

50	Annick	**Wilmotte**	Baseline on cyanobacterial biodiversity of Svalbard asessed by pyrosequencing	P-E-08
51	Annick	**Wilmotte**	The BCCM/ULC culture collection to conserve, document and explore the polar cyanobacterial diversity	P-E-09
52	Olga	**Baulina**	The biodiversity and plasticity of symbiotic *Desmodesmus* spp. (Chlorophyceae) from a subarctic sea	P-F-01
53	Kateřina	**Kopalová**	Diatoms from the maritime Antarctica region; extreme endemism in Antarctica	P-F-02
54	Kateřina	**Kopalová**	An intriguing species of *Eunotia* (Bacillariophyta) from Gough Island (Tristan da Cunha archipelago)	P-F-03
55	Kateřina	**Kopalová**	Revision of the genus *Nitzschia* in the maritime Antarctic Region	P-F-04
56	Kateřina	**Kopalová**	The genus *Luticola* D.G.Mann (Bacillariophyta) from the McMurdo Sound Region, Antarctica, with description of four new species	P-F-05
57	Ľubomír	**Kováčik**	Exposition of various stress conditions on filamentous green alga *Klebsormidium* (Streptophyta)	P-F-06
58	Jana	**Kvíderová**	New record of cryoseston on Olympus Mt., Greece	P-F-07
59	Dial	**Laughinghouse**	Unique diversity of marine microbial eukaryotes in the High Arctic	P-F-08
60	Roksana	**Majewska**	Epiphytic diatom communities from Terra Nova Bay and Cape Evans (Ross Sea) - a synthesis	P-F-09
61	Tomomi	**Nakashima**	Temporal and spatial variations in pigment compositions of snow algae in Mt. Tateyama in Toyama prefecture, Japan	P-F-10
62	Linda	**Nedbalová**	Identity and ecophysiology of coccoid green alga dominating in ice-covered lakes on James Ross Island (NE Antarctic Peninsula)	P-F-11
63	Eveline	**Pinseel**	Hidden diversity: multiple Arctic and Antarctic lineages in the cosmopolitan diatom *Pinnularia borealis*	P-F-12
64	David	**Ryšánek**	Diversity and spatial capacities of Arctic terrestrial algae	P-F-13
65	Klemens	**Weisleitner**	Advances in laser-induced fluorescence emision technology (L.I.F.E.) and preliminary microbial data from an Antarctic glacier	P-H-01

Abstracts

Session A
Polar/alpine microbiology and environmental change

Keynote lecture KN-A

THE IMPACT OF LARGE GRAZERS ON THE RESPONSES OF SOIL MICROBIAL COMMUNITIES TO WARMING AND INCREASED NITROGEN AVAILABILITY IN SUB-ARCTIC TUNDRA

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Arctic tundra soils store large quantities of the global organic carbon pool and hence there is considerable interest in understanding how this carbon will respond to environmental changes caused by a warming climate. Regulators of SOM decomposition are, however, complex and it is not well known how different feedback mechanisms will influence carbon and nitrogen cycling in the future climate. One of the key limitations in understanding the drivers of SOM decomposition is our still very poor understanding of the microbial communities, how the environmental factors drive the community structure and function and how the microbial community structure influences the C and N cycling.

Large grazers, particularly reindeer, influence the tundra ecosystems drastically through faeces, trampling and selective grazing leading to changes in vegetation, soil microclimate, nutrient availability, and quality and quantity of litter and soil carbon. All these factors influence the carbon and nutrient cycles with a consequence that long-term reindeer grazing may diminish the effects of warming on ecosystem net carbon balance. To understand links between grazers and microbial responses to global change, we have conducted field and laboratory experiments in soils from Raisduoddar, Norway. A pasture rotation fence divides the area to a lightly grazed winter pasture with lower N availability, lower soil temperature and shrub dominated vegetation and to a highly grazed summer pasture with high N availability, higher variation in soil temperature and grass dominated vegetation. The effects of experimental warming and fertilization were characterized by Ion Torrent sequencing of bacterial 16S rRNA amplicons, soil respiration, extracellular enzyme activities and soil physico-chemical analyses.

The-experimental treatments had significant effects on the microbial communities but the magnitude and/or direction of the effects depended on the grazing intensity. Warming had little influence on the microbial community structure or activities in the heavily grazed tundra. In the lightly grazed tundra, however, warming shifted the bacterial community significantly and increased e.g. the abundance of several *Actinobacteria* taxa. Fertilization increased the proportion of carbohydrates in soil with a concomitant increase in microbial activities related to carbon degradation. In lightly grazed tundra fertilization increased several OTUs related to *Actinobacteria,* while in heavily grazed tundra fertilization was linked especially to the increase in taxa related to the *Dyella/Rhodanobacter* group (*Gammaproteobacteria*). Similarly as reported for warmer soils, fertilization favoured copiotrophic species (*Actinobacteria, Gammaproteobacteria*) while oligotophic taxa (e.g *Acidobacteria, Verrucomicrobia*) decreased. The results indicate, however, that in the fennoscandian tundra, the effects of fertilization were mediated through changes in organic matter quality rather than N availability directly. Put together, our results indicate that reindeer mediated changes in the soil chemical composition and microclimate have a profound influence on the structure and functional adaptation of soil microbial communities. This role of large grazers may be a key mechanism determining the impact of warming in carbon fluxes in the tundra.

KEYWORDS: MICROBIAL COMMUNITIES, ARCTIC TUNDRA, ENZYME ACTIVITIES, NEXT-GENERATION SEQUENCING, GRAZING

Lecture A-01

MICROBIAL SUCCESSION FROM ICE TO VEGETATED SOILS IN THE HIGH ARCTIC

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Glaciers in the Northern Hemisphere are retreating and their forefields present a unique opportunity to investigate the initial phases of soil weathering/formation and microbial succession in terrestrial cold habitats. In Arctic environments, the relative importance of primary autotrophic microbial colonisers (e.g. cyanobacteria and chemolithotrophs), input of allochthonous sources and recycling of ancient organic carbon during the initial phase of soil establishment is still debated. In glacial forefields, microbes that have colonised both glacial surfaces and subglacial debris may provide an important inoculum for the development of microbial communities. In this study, during the summer in 2013, we collected soil samples along three replicated transects from the margin of the Mitdre Lovénbreen (Svalbard, Norway). These transects represent a chronosequence between 0 and ~ 2000 years dated based on geomorphological data. We cross-compared these soil samples with samples from the glacial surface and basal sediments. Analyses of microbial community composition (ssu rRNA gene), activity (C fluxes, N-fixation, and C utilisation) as well as bulk nutrient (e.g. N, P) and carbon budgets (organic and inorganic) in the soils show some similarities with previous studies conducted in the Alps, where there is an increase in a variety of microbial proxies, such as microbial enzymatic activity, respiration and alpha diversity, in relation to years of exposure after glacial retreat. Our study shows a clear succession of microbial communities with age where communities in soils previously overridden by the ice (strongly represented by members of the Betaproteobacteria such as the genus *Thiobacillus*) are important colonizers of new exposed soils up to 5 years after glacier retreat. Thereafter, presence of typical soil communities such as Acidobacteria and certain members of Actinobacteria and Alphaproteobacteria (e.g. *Sphingopyxis*) become more prevalent. Despite the fact that there is a strong autotrophic presence within the microbial community along the chronosequence, a combination of geochemical, activity and modelling data reveals a dependence of allochthonous and vegetation growth to explain organic carbon accummulation. Nevertheless, the microbial succession observed in our chronosequence gives insights of feedback mechanisms between geochemistry and microbial colonisation during soil development after glacial retreat which could estimulate release of nutrients and promote the initial stages of plant colonisation.

KEYWORDS: GLACIAL FOREFIELDS, MICROBIAL SUCCESSION, DIVERSITY, SOIL, CHRONOSEQUENCE, NUTRIENTS, CARBON BUDGET

Lecture A-03

BACTERIAL UTILIZATION OF CARBON AND NITROGEN AT SUBZERO TEMPERATURES IN TUNDRA SOILS

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We seek to delineate the active bacterial community in Arctic tundra soils and discern their roles in C and N metabolism under subzero conditions. By understanding these processes in frozen soil systems, we hope to better predict the extent of C and N released as greenhouse gases which subsequently contribute to climate change. Our overall hypothesis is that Arctic tundra harbors hypo-psychrophilic microorganisms, akin to permafrosts, which grow and metabolize organic matter at subzero temperatures. It is established that microbes in frozen Arctic soils can continue to mineralize organic carbon at significant rates. There is, however, a paucity of information of the chemical forms of C and N metabolized by specific psychrophilic microorganisms. The divergent life-styles of different microorganisms will be reflected in their ability to function in frozen soils and in their responses to environmental perturbations, leading to seasonal dynamics of activities. Utilizing a stable isotope probing (SIP) approach we seek to understand how bacterial communities modulate their response to variations in temperature and pulses of substrate input and availability. A suite of ^{13}C (acetate, cellobiose, complex cellular carbon algal digest) and ^{15}N (nitrate, ammonia, urea) substrates were fed to soil microcosms and incubated at temperatures of +4, 0, -4, and -15 °C. The “heavy” and “light” DNA profiles of each microcosm were analyzed and compared in order to identify the active bacterial community members at different temperatures, as these microorganisms assimilate the heavy isotopic substrate, synthesize biomolecules, and replicate their genomes. Shifts in the active bacterial communities from the initial resident communities were detected by community fingerprints using 16S rRNA gene terminal restriction fragment length polymorphism (T-RFLPs) analysis. Different community members were active on the different C and N substrates. Furthermore, in permafrost, we demonstrated that some members of the bacterial community were active across temperatures of 0 to -20°C, while, intriguingly, others only synthesized DNA within a narrow subzero temperature range. This implies that small subzero temperature changes may lead to broad changes in the active microbial community, which may have consequences for biogeochemical cycling in seasonally or permanently frozen soils.

KEYWORDS: STABLE ISOTOPE PROBING, C AND N METABOLISM, TUNDRA, SUBSZERO

Lecture A-04

ARCTIC BACTERIAL SEA ICE COMMUNITIES AFFECTED BY GLOBAL WARMING

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Currently climate change is faster and more severe in the Arctic than in most of the rest of the world. A reduction in the extent and thickness of sea ice as well as of multiyear sea ice is clearly visible since years. Whether these dramatic changes also affect the bacterial communities of sea ice, melt ponds and seawater underneath the ice was investigated by comparing results of studies conducted in 1997, 1999, 2000 and 2011. Potential changes of the bacterial communities were analysed by: total counts, viable counts and secondary production at two temperatures, diversity, and taxonomy. Of course, such a comparison is limited by the fact that microbiological and molecular biological methods changed over the time. Further, sea ice is a drifting ecosystem and the surface water masses in the Arctic Ocean are not uniform. While the earlier studies concentrated more or less on regions influenced by Atlantic water the route of the 2011 cruise lead to the Central Arctic Ocean thus samples of Atlantic as well as Pacific influenced water could be studied.
In the Atlantic influenced area higher secondary production rates, determined by means of thymidine incorporation, were obtained in comparison to the Pacific influenced area. Bacterial activities of the sea ice samples in the Atlantic section exceeded in most cases those of melt pools while in the Pacific section melt pools and upper ice samples were more active than the deeper ice layers. However, especially in the Atlantic influenced area, a high percentage of the melt pools were covered by big yellow/orange aggregates. Such amounts of visible organic matter were not observed during the cruises in 1997, 1999 and 2000. Overall a tendency became obvious that with global warming productivity increased in melt ponds and water beneath the ice but decreased in sea ice. An adaptation to warmer temperatures became visible in all three habitats on the basis of secondary production investigations and viable count determinations at 1°C and 22°C. Further, specific types of *Betaproteobacteria* and actinomycetes that were found to be indicative for freshwater ponds during 1999 and 2000 occurred in 2011 quite frequently in bottom sea ice sections and bacteria indicative for the oligotrophic water column (SAR11) made up nowadays a noticeable percentage in sea ice and even melt ponds. The dominance of *Gammaproteobacteria* is obviously taken over by members of the *CFB*-group. *Alphaproteobacteria* played a more pronounced role in the area influenced by Pacific Water.

KEYWORDS: ARCTIC OCEAN, BACTERIAL COMMUNITIES, SEA ICE, MELT PONDS, CLIMATE CHANGE

Lecture A-05

MICROBIAL MAT COMMUNITIES ALONG ENVIRONMENTAL GRADIENTS IN PERENNIALLY ICE-COVERED LAKE FRYXELL, ANTARCTICA

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Perennially ice-covered, meromictic lakes in the McMurdo Dry Valleys, Antarctica, are useful models to study the relationship between microbial communities and environmental variables. They have rich benthic cyanobacterial mat accumulations and stable stratification of physical and chemical conditions. In Lake Fryxell, we were able to evaluate for the first time the microbial communities within a steep oxygen gradients ranging from 20 mg L^{-1} to undetectable. We were able to obtain a detailed understanding of the benthic macroscopic mat morphologies and assemblages in Lake Fryxell using a combination of *in situ* oxygen measurements, pigment analysis, morphological descriptions and next generation sequencing of 16S rRNA gene bacterial community. We idenfitied three macroscopic mat morphologies such as "cuspate pinnacles" with complex topography in the upper hyperoxic zone, dominated by phototrophic filamentous, phycoerythrin-rich cyanobacteria attributable to the genus *Leptolyngbya*, together with a diverse assemblage of pennate diatoms and heterotrophic Proteobacteria and Bacteriodes; a less topographically complex "ridge-pit" mat, increasingly rich in diatoms immediately above the oxygen limit with a similar heterotrophic bacterial assemblage; flat prostrate mats in the upper anoxic zone, with dominance of the cyanobacterium *Phormidium pseudopriestleyi* based on 16S rRNA gene phylogenetic analysis, and the diatom *Diadesmis contenta*. The *P. pseudopriestleyi* mat created an oxygen-rich micro-habitat in the upper part of the mat in an otherwise euxenic region of the lake during the austral summer conditions. Within prostrate mats anoxygenic phototrophs became more abundant based on an increase in green sulphur bacteria 16S rRNA gene sequences and B-chlorophyll-a. Archaea such as Crenarchaeota and Parvarchaea were also identified with low relative abundances in all mats. The results suggest that oxygenic and anoxygenic microbial communities formed assemblages in niche-like locations, and the macroscopic mat morphologies are likely due to cyanobacterial composition as a result of habitat conditions such irradiance, oxygen and sulphide/sulphate concentrations.

KEYWORDS: CYANOBACTERIA, MICROBIAL MAT, LAKE, ANTARCTICA, 16S RRNA GENE

Lecture A-06

MICROBIAL PLANKTONIC COMMUNITIES AS ENVIRONMENTAL INDICATORS IN A TIERRA DEL FUEGO PEAT BOG

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INTRODUCTION

Peatlands are key ecosystems which store 30% of all C in the soil and 10% of the total freshwater volume on Earth. Only a small proportion of these wetlands are located in the Southern Hemisphere; and opposedly to their Northern Hemisphere counterparts, their ecology and diversity has been poorly studied. The insular Province of Tierra del Fuego (Argentina) encompasses a vast area of pristine peatlands (ca. 27000 km^2), many of which occupy glacial valleys between the Andean ridges, are typically dome-shaped and ombrotrophic (fed only by precipitation) and host many small pools. At Rancho Hambre peat bog, we aimed at investigating the influence of climatic, topographic and morphometric features on the abiotic environment of the pools, and how this shaped the structure and dynamics of planktonic communities.

MATERIALS AND METHODS

Eight samplings were carried out in October, December, February and April 2008-2010 in five pools representing distinct morphometric features. An automatic weather station was located in the area. Abiotic features (water temperature, conductivity, pH, transparency, dissolved oxygen, total hardness, DOC, total N and P, NO_3-N, NH_4-N and PO_4-P) were measured. Abundance and biovolume of heterotrophic bacteria (HB) and flagellates (HF), picophytoplankton (PP) nano+microphytoplankton, ciliates and mesozooplankton were measured on quantitative samples. Also, species richness, taxonomic composition and diversity were analyzed on qualitative samples of nano+microphytoplankton, ciliates and mesozooplankton (Quiroga et al. 2013). In 2012, the molecular diversity of the prokaryotes and the smaller (<3 µm) fraction of the eukaryotes of these same pools were studied by means of high-throughput sequencing (Quiroga et al. 2015, Lara et al. 2015).

RESULTS

Topographic variations resulted in a low hydrological connectivity, which largely accounted for the strong environmental differences found among pools. These could be classified into ombrotrophic (softer, more acidic) and minerotrophic. Their morphometry played a key role by modulating seasonal changes in water temperature and hydrological stability. In spring, the structures of the planktonic communities were similar, whilst in late summer there were significant differences in the abundance and biomass of the different trophic compartments among small, shallow water bodies and large ones. Results of a canonical correspondence analysis ascribed these to pool size-driven patterns of water temperature variation (Quiroga et al. 2013).

Analysis of the taxonomic composition of nano+microphytoplankton (Mataloni et al. 2015) and ciliates (Küppers et al. in prep.) coincided in low values for Jaccard index among pools. Despite such differences, phytoplankton of minerotrophic pools was typically richer in desmids, diatoms and Chlorophytes, while that of ombrotrophic ones was richer in cyanobacteria. These communities underwent paralell structure changes over time.

The community composition of the smallest size fraction (<3 µm) of eukaryotic plankton was analysed using GUniFraC and separated minerotrophic and ombrotrophic sites (Lara et al. 2015). The 5% best indicators for both environments were searched using an IndVal analysis. Among these, autotrophic taxa were more common in minerotrophic environments, whereas mixotrophic taxa represented best ombrotrophic water bodies.

Bacterioplankton communities were diverse (72% of rare sequences) yet widely dominated by only 10 OTUs belonging to the Proteobacteria, Actinobacteria, Bacteroidetes and Verrucomicrobia, which represented 53% of sequences. PERMANOVA analyses showed that community structure was largely explained by differences in hydrological connectivity, pH and nutrient status (ombro/minerotrophic pools) (Quiroga et al. 2015).

DISCUSSION

The results pertaining all studied communities revealed a strong underlying pattern supporting the characterization of pools according to their size and minero/ombrotrophic character. Overall differences in the structure of trophic compartments became more evident with the onset of the growth season. Remarkably, molecular diversity spatial patterns for both small-sized eukaryotes and prokaryotes coincided with those detected for larger planktonic communities studied by traditional morphology-based taxonomy. In general, ombrotrophic pools showed significantly poorer communities, suggesting stronger environmental filtering operating in these pools.

KEYWORDS: PEAT BOGS, PLANKTON, COMMUNITY STRUCTURE, ENVIRONMENTAL INDICATORS, MOLECULAR DIVERSITY

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Lecture A-07

ENVIRONMENTAL PRESSURE AND VARIATION OF FUNGAL BIODIVERSITY IN ROCK MICROBIAL COMMUNITIES OF NORTHERN VICTORIA LAND (ANTARCTICA)

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Microbes in Antarctica, more than in other continents, are predominant and rocks, in the ice-free areas, represent the main substratum for colonization. Rock colonizers spread in the ice-free areas and, when conditions become too harsh to allow active life on the surface, the endolithic niche offers more buffered conditions; therefore, the endolithic lifestyle represents a borderline adaptation and a last chance for survival (Friedmann, 1982).
Polar ecosystems are very sensitive to Climate Change; over the past 50 years regions of Antarctica and sub-Antarctic islands have experienced some of the most rapid increases in mean air temperatures on Earth (Steig et al. 2009). The Antarctic endolithic communities, living at the edge of their biological potential, are particularly sensitive to any external variation. The establishment of more permissive conditions and subsequent introduction of competitive alloctonous species may cause the extinction of some highly adapted autoctonous components of the communities (Selbmann et al. 2012). For these reasons it is of utmost importance to investigate the amplitude and preserve this threatened and mostly still unknown biodiversity before any climatic variation leads to possible extinction.
Moreover, responses of these communities in term of distribution, species richness and biodiversity variation under different environmental stress is unknown at present. Yet, a deeper understanding of these Antarctic ecosystems may allow identifying changes and give clues to monitoring or predict the effect of any future variation due to Climate Change.
Recently a survey was performed to map the distribution of lithic colonization in Victoria Land to define the geographic borderline for life and distribution of epi- and endolithic colonization. The colonization followed the climatic variation, epiliths prevailed in coastal sites while chasmoendoliths and cryptoendoliths towards the inland sites. Typical cryptoendolithic colonization was exclusive of sandstone; multivariate analysis revealed the pivotal role of different rock types; where sandstone was present lithobionts were pushed towards harsher conditions (Zucconi et al. 2014).
In the present study it was analyzed the variation of biodiversity in the endolithic communities related to environmental parameters of altitude, sea distance and rock substratum. The study was focused on fungal component first as one of the main settlers of the communities. A selection of 72 rock samples were analyzed, chosen as representative of the total sampling. Total biodiversity was studied using DGGE approach and data were analyzed in terms of biodiversity in relation to environmental parameters.
Pareto Lorenz distribution curves were used to estimate evenness and functional organization within the fungal community. Results revealed the presence of few dominant species indicating a high degree of organization and specialization but high vulnerability to external changes; this means that a long recovery time might be required after intense perturbing events, such as alien species invasion.
No linear correlation was found among different biodiversity indexes and environmental parameters besides, they appeared significantly related when analyzed with a Non Metric Multi-Dimensional Scaling (NMDS).
Rock porosity seems to influence richness of biodiversity and in a certain extent a proper porosity allows colonization towards high altitude and sea distances; yet, when airspaces reduces, amplitude of biodiversity is mostly dependent to altitude and sea distance.
Sandstone, as the most porous rock, allows a more efficient colonization of endoliths and influences the richness of biodiversity.

KEYWORDS: ANTARCTICA, CLIMATE CHANGE, DGGE, ENDOLITHIC COMMUNITIES,

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Lecture A-08

CHANGES IN BACTERIAL COMMUNITY COMPOSITION ALONG A TURBIDITY GRADIENT IN RECENTLY-FORMED LAKES IN SW GREENLAND

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Receding glaciers and ice masses are among the most prominent signs of global climate change and have created many new proglacial lakes where topology is suitable. Those highly turbid proglacial lakes are dominated mainly by microbes and predation losses by grazing or viral attack are probably low. Little is known, on how the structure of the planktonic bacterial community changes with decreasing turbidity, when lakes loose the connectivity to the glacier or ice-sheet. In this study, we sampled six lakes along a turbidity gradient formed by the retreat of the Greenland ice sheet (GIS) within the last decades. The turbidity gradient was associated to a strong shift in DOM properties. Patterns in diversity and bacterial community composition were analyzed using NGS of the 16S rRNA gene. Alpha-diversity was not influenced by turbidity, but community composition exhibited significant differences along the turbidity gradient. Lakes most strongly influenced by GIS melting had some unique phylotypes which can be considered as 'turbid specialists'. Interestingly, *Limnohabitans,* an active and widespread bacterial taxa in lake communities, exclusively occurred in lakes with low turbidity. Glacier runoff shared only ca. 8.4% of the total bacterial richness found in the lakes and had a large number of unique phylotypes, suggesting that either many taxa are not able to establish in the recipient lakes or that there is a temporal dynamic component. Overall, our data suggest that newly-created lakes have a striking high bacterial diversity with specialized taxa being generally less represented.

KEYWORDS: ARCTIC, CLIMATE CHANGE, GLACIER RETREAT, LAKES, BACTERIA

Poster A-01

METHANE OXIDATION AND METHANE DISTRIBUTION AROUND THE LENA DELTA, SIBERIA, RUSSIA

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The Lena River is one of the biggest Russian rivers draining into the Laptev Sea. Due to predicted increasing temperatures the permafrost areas surrounding the Lena will melt at increasing rates. With this melting high amounts of carbon, either organic or as methane will reach the waters of the Lena and the adjacent Laptev Sea. As methane is an important green house gas its further fate in the Lena Delta is of uttermost importance. Methane oxidation by methanotrophic bacteria is the only biological way to reduce methane concentrations. However, the polar estuary of the Lena River is a challenging environment, with strong fluctuations in salinity and temperature. We determined the activity and abundance of aerobic methanotrophic bacteria (MOB), as well as the methane distribution and other abiotic parameters. Activity was determined with 3H-CH4 as radioactive tracer and abundance was determined with quantitative PCR.

Methane concentrations were rather low (41 ± 44 nM), as well as methane oxidation rates (1.1 ± 1.6 nM/d). In polar water (cold and saline) highest activities were found, whereas the highest abundance of MOB was in surface waters. The relation between methane turnover and abiotic factors will be used to characterize the eco-physiology of these polar and estuarine methanotrophs.

Poster A-02

MICROBIAL COMMUNITY DEVELOPMENT ON DEGLACIATED SOILS IN HIGH ARCTIC (SVALBARD) IN COMPARISON TO SUB-ARCTIC CONTINENTAL REGIONS (SCANDINAVIA)

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Glacier retreats represent valuable possibilities to study primary succession of organisms on land and ecological consequences of global warming. We aimed to compare development of bacterial community composition along glacier retreat in High Arctic (Central Svalbard) and in subarctic alpine regions in Scandinavia. In Svalbard, we sampled 3 different glacier forefields (Hørbyebreen (H), Ferdinandbreen (F), Ragnarbreen, (R)) in the vicinity of Petuniabukta Bay (Billefjorden, central Svalbard). Along the glaciers retreats, we defined 4 successional stages, two younger (early (1) and middle (2)) and two older (late (3) and climax (4)) than LIA moraine in Svalbard forefields. In Scandinavia, continental glacier retreats – Storglaciären (SG, Sweden) and Midtdalsbreen (MB, Norway) were investigated. Here, we sampled 6 successional stages, defined as fresh (barren) soil (0), initial (1), early (2), middle (3), middle-late (4), and climax (5) stages. The aim of the study was to compare the successional trends of microbial communities in two different ecosystems (Svalbard vs. Scandinavia). We aimed to identify microbial taxa discriminating successional age and soil development as well as reveal the environmental predictors behind. Soil bacterial communities were studied by 454 pyrosequencing of V6 region of 16S rRNA.

In Svalbard chronosequence, bacterial community development was driven by successional age, locality and concentration of phosphates. Db-RDA showed the discrimination of younger stages (1,2) by PC1, explaining 21.1% of total variability. Second axis discriminated R transects from both F and H. Total C (TC), total N (TN), and microbial biomass (Cmic) increased along the successional age, while TP do not. Amount of TC differed among chronosequences, as well as Cmic. Soil pH was stable, decreasing only in F chronosequence. C-to-N ratio was consistent along R and H chronosequences. In Svalbard, the primary succession of bacterial communities followed the trend of the dominance of Actinobacteria, Proteobacteria in young soils to the dominance of Acidobacteria and Chloroflexi in developed soils.

In Scandinavia, bacterial community development is driven by soil pH, successional age, and locality. Db-RDA showed differences in successional stages and both chronosequences. PC1 axis explained 23.5% of total variability and discriminated fresh and initial stages from older ones and MB transect from SG. The differences in bacterial communities between MB and SG chronosequences are likely influenced also by availability of nutrients, which are given by different geology of localities. Granite is dominating in MB and amphibolites are main bed rocks in SG. In MB chronosequence, there is significantly higher TC, TN, and total P (TP), than in SG. Cmic increases with successional age and with the increase of concentration of nutrients (TC, TN, TP), being higher in MB only in young stages (0-2) in comparison to SG. In contrary, soil pH dropped from neutral to highly acidic. The soil development was characterized also by the significant increase in C-to-N ratio. This was extremely low in young stages, indicating the C limitation in the pioneer microbial communities. In Scandinavia, the youngest stages were dominated by Proteobacteria, Firmicutes and Gemmatimonadetes, while climax stage was characterized by Acidobacteria, Armatimonadetes, Chlamydiae, Planctomycetes, and Verrucomicrobia.

In conclusion, we assume that development of soil bacterial communities differed between Svalbard and Scandinavia, which might be mainly driven by soil pH and availability of nutrients. In both ecosystems shift from pioneer bacteria represented by Proteobacteria to Acidobacteria dominated communities were observed.

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KEYWORDS: SOIL DEVELOPMENT, NEXT GENERATION SEQUENCING, BACTERIAL COMMUNITIES, CHRONOSEQUENCES, NUTRIENTS

Poster A-03

TERRESTRIAL INVERTEBRATES ALONG A GRADIENT OF DEGLACIATION IN SVALBARD: RELATION TO MICROBIAL COMMUNITIES

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The changes of terrestrial invertebrate populations along three transects of deglaciation have been studied in Petuniabukta Bay (Billefjorden, central Svalbard). Populations of Rotifera, Tardigrada, Nematoda, Enchytraeidae and Collembola have been studied with respect to quantitative parameters of microbial community studied by 454 pyrosequencing method.

Glacial forehead of three glaciers retreats fastly during last decades. Sites of about 10 years old are one extreme of gradient in contrast of well developped tundra communities on sea terraces of about 10 000 years old. However the range of altitudes is less than 200 m, strong gradient of development is evident along the sites and extremity of arctic environment cause very different conditions in case of temperature and water availability (Kaštovská et al. 2005).

Strong changes of microbial as well as invertebrate populations on gradients are evident and well developped. Nematods as most abundant group reached abundance from 13 to 376 ind 10 cm^{-2}, rotifers from 0 to 78 ind 10 cm^{-2}, tardigrades from 0 to 58 ind 10 cm^{-2}. Quantitative analyses of populations show, that sampling sites differ on transects, as well as transects differ mutually. Although populations generaly increase from young to old plots in case of abundance and diversity, such trend is not universal and in many cases are maxina in younger plots across groups. the most typical looks to be Hørbybreen valley, where the rotifers, tardigrades as well as nematodes are most abundant in third position from the glacier forehead, In total 21 species of bdelloid and monogont rotifers were found if most abundant was *Encentrum arvicola*, *E. lutra*, *Macrotrachela* sp. and *Habrotrocha rosa*.

ACKNOWLEDGEMENT: The study was supported by grants No. COST-LD13046 and No. LM 2010009 of the Ministry of Education, Youth and Sports of the Czech Republic.

KEYWORDS: SOIL DEVELOPMENT, NEXT GENERATION SEQUENCING, INVERTEBRATE COMMUNITIES, CHRONOSEQUENCES

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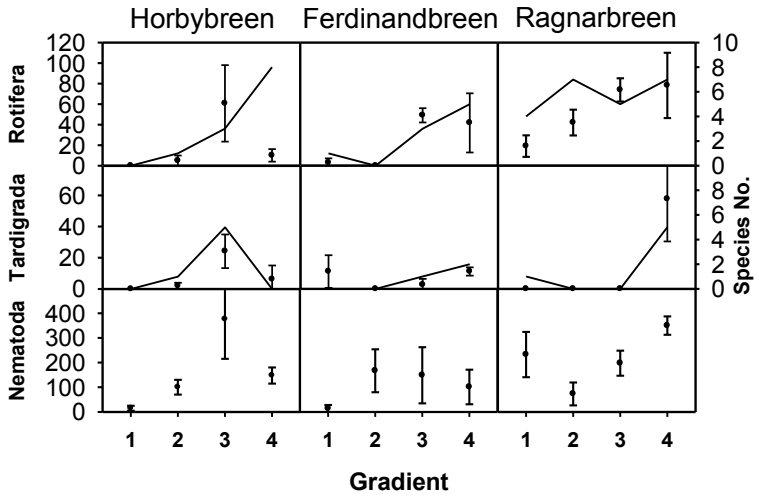

Fig. 1. Changes of abundance and diversity of three invertebrate groups along the gradients

Poster A-04

MICROBIAL BIOMASS AS AN INDICATOR OF CARBON LOSSES FROM SUBARCTIC TUNDRA SOILS IN CHANGING ENVIRONMENT

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Soils of high latitude ecosystems are experiencing an increase in temperature and precipitation in a result of recent climate change. Subsequent permafrost thawing will possibly alternate conditions in active layer forming either i) well-drained areas with aerobic soils or ii) inundated soils upon impermeable layers and thermocarst lakes following collapse of soil profile, both subjecting the uppermost soil to anaerobic conditions. Temperature together with O_2 availability is expected to affect microbial mineralization of soil organic matter (SOM) resulting in C losses as well as to predetermine the form in which C will be released (CO_2 or CH_4).

Major proportion of SOM in East-European subarctic tundra is stored in raised peat plateau (PP) complexes despite their rather minor landscape coverage compared to extensive mineral tundra (MT). Peat plateaus are often dotted with bare ground features affected by frost actions called peat circles (PC) with distinct N cycling resulting in N_2O emissions (Repo et al. 2009). These three habitats have the major impact on C and nutrient balance of the study area and therefore have been subjected to several field studies. Recent laboratory experiment aims to asses the role of environmental factors (temperature, O_2 availability), microbial biomass, composition of microbial community, substrate and nutrient availability in C release from subarctic soils.

We incubated homogenized active layer (2-15 cm depth) of the PP, PC and MT soils (locality Seida, Komi Republic, Russia; 62°57´E, 67°03´N) in a factorial set-up of three temperatures (4, 12, 20°C) and two moisture/O_2 levels (80% water holding capacity [WHC] – aerobic, 100% WHC – anaerobic). Gas exchange (CO_2, CH_4, O_2 and N_2O) in the incubation vessels was monitored over the time and following soil parameters were determined at the beginning and the end of the incubation: extractable N and P forms, total C and N, extractable organic C and its quality (UV spectroscopy), microbial biomass, microbial community composition (q-PCR, pyrosequencing) and extracellular enzyme activities.

The highest C mineralization rates per gram dry weight were attributed to the C-rich PP soil with relatively labile substrate and intermediate nutrient availability. Lower rates were detected in MT soil which was poor in C and nutrients but had the most labile substrate. Strongly humified organic PC soil with the highest proportion of aromatic substrate and highest nutrient availability showed the lowest C mineralization.

Our results suggest that the size of microbial biomass plays the key role in C release from different soils which is in accordance with similar study conducted with cryoturbated Siberian soils (Čapek et al.). The biomass size is not a simple function of extractable C but depends strongly on the quality of C substrate. Carbon quality also affects temperature response of C mineralization and its decrease under anaerobic conditions. C mineralization in PP soil was the most sensitive to temperature and O_2 availability while PC soil responded to changed environmental variables only with slight differences in C mineralization. Methanogenesis contributed to the C mineralization only in MT soil under anaerobic conditions representing up to 43% of total C loss. In contrast, PP and PC released C in anaerobic conditions solely in form of CO_2. Methanogenesis in these peat soils was hampered either by lack of suitable substrate for methanogenes or possibly by slow evolution of methanogene community.

Climate models usually estimate the future C losses from soil on a basis of total C or extractable C. Here, we infer that C quality would need to be taken into account when predicting C losses from northern soils in changing environment.

KEYWORDS: SUBARCTIC TUNDRA, SOM MINERALIZATION, TEMPERATURE SENSITIVITY, O_2 AVAILABILITY, MICROBIAL BIOMASS

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Poster A-05

SNOW ALGAE INCREASES SNOWMELT: RESULTS OF A MANIPULATIVE EXPERIMENT ON THE HARDING ICEFIELD, ALASKA

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Snow algae are an important microbial component of coastal glaciers in Alaska where they inhabit the equilibrium line altitude (ELA) between the zone of accumulation and ablation. The ELA roughly marks the snow line where low-albedo bare ice contributes substantially more meltwater than high-albedo snow. Like all organisms, snow algae require liquid water for growth and reproduction, yet are unable to adhere well to bare ice; hence snow algae prefer wet, melting snow habitats. Here we show experimentally that snow algae also increase snow melt. We established 21 experimental blocks in a 0.5 km^2 area with four treatments per bock (bleach, fertilizer, water, and control) and measured melt from May through August. In addition, we sampled snow algae to determine if our experimental treatments did effectively control algae density and biomass. We found that there was no difference in melt among treatments from May — July when algae were not visible on the surface of the snow, but that during the month of July when algae bloomed that the fertilizer treatments had both significantly more melt and more algal biomass than controls. Similarly bleach plots had significantly less melt and less algal biomass than controls. By August snow in the study area had melted to ice and there were no significant differences among treatment types in melt rate. Using a spectrometer, algal counts and biomass estimates, and Landsat 8 satellite imagery we built a spatial model of the contribution of algal biomass to meltwater across the Harding Glacier. These results show experimentally that snow algae is involved in a positive feedback loop with snow melt, such that more melt increases algae biomass and algae biomass increases melt and that the distribution of melt due to algae can be very extensive.

Poster A-06

SPATIAL AND TEMPORAL INFLUENCES ON ARCTIC SOIL MICROBIAL COMMUNITY STRUCTURE

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INTRODUCTION

Arctic heathlands are home to uniquely adapted biodiversity and a globally relevant carbon reservoir. Predicted atmospheric warming could increase the vulnerability of soil carbon to microbial decomposition and exacerbate feedbacks to climate change. The sensitivity of plot-scale experiments simulating climate change impacts on soil microbial communities (e.g. Johnson et al. 2002; Rinnan et al. 2007) needs to be evaluated within the context of spatial and temporal variation across the ecosystem. We investigated microbial communities of Arctic heathland soils from northern Sweden across a range of sites and scales. 16S rRNA gene T-RFLP and amplicon sequencing were used to determine spatio-temporal variation in soil microbial communities and under snowmelt manipulations.

MATERIALS AND METHODS

During May and July 2013 soil cores were collected from two sites in a sub-Arctic heathland at Abisko, Sweden. Four idential 50 m transects, each at a 90^{o} rotation from the origin, were sampled at repeated varying-lag distances. Genomic DNA was extracted from freeze-dried soil and the bacterial community structure characterised by T-RFLP fingerprinting. A sub-set of samples were subject to Ion Torrent semiconductor sequencing for further analysis of bacterial diversity. Community networks were constructed from OTUs processed in QIIME using Cytoscape, and predictions of metagenomic function carried out with PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). During spring, bacterial and fungal communities were also sampled from heathland soils where overlying snow cover had been perturbed by promoting or delaying the rate of snow melt.

RESULTS

T-RFLP did not reveal spatial structuring of communities at scales below 100m at any site or season. However, a strong seasonal shift in community structure was apparent between spring and summer. Amplicon sequencing supported evidence of temporal changes in bacterial diversity, specifically a decline in *Betaproteobacteria* dominance which was countered by greater abundance of *Acidobacteria* and *Alphaproteobacteria* between seasons (Figure 1). Predicted metagenomes of community function revealed the most abundant KEGG pathways by copy number count related to environmental information processing and genetic information processing, with a trend for increases in pathways associated with energy uptake and microbial growth and repair during summer. Network analyses revealed two seasonal keystone taxa: a spring betaproteobacterial sub-network centred upon a *Burkholderia* OTU, with reconfiguration to a summer sub-network centred upon an alphaproteobacterial OTU (Figure 2). Finally, perturbation of spring snow cover revealed a slight trend for greater bacterial diversity, in addition to significant ($p < 0.05$) reductions in soil fungal biomass, in response to earlier snow melt.

DISCUSSION

Findings suggest that spatial structuring effects may not confound comparison between treatments within plot-scale experiments, but larger distances (>100 m) and seasonal processes may have significant influences. Changes in community structure observed between spring and summer suggest a shift from r- to K-selected taxon dominated communities, potentially influencing in silico predictions of functional potential. Coupled with temporal changes in KEGG pathways, this infers the notion that the spring bacterial community is formed by rapid growth and occupancy of a narrow niche space by copiotrophs within the thawing soil, whilst the summer community is configured to take advantage of a diversified range of niches and inputs at the time of peak aboveground biomass. In addition, the importance of two season-specific keystone taxa identified by 16S rRNA gene sequencing suggests that stability of Arctic heathland soil bacterial communities may be vulnerable to seasonal perturbations disproportionally affecting individual taxa. However, no clear repsonse was detected by T-RFLP fingerprinting of heathland soil bacteria after melting of the snowpack had been manipulated, which may indicate that the most abundant soil microorganisms are resilient to short-term perturbations to certain seasonal processes.

KEYWORDS: MICROBIAL ECOLOGY, ARCTIC SOIL, CLIMATE CHANGE, T-RFLP, ION TORRENT

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		Spring								Summer							
		Site 1				Site 2				Site 1				Site 2			
		1MN495	1ME495	1MS495	1MW495	2MS495	2MW495	2MN495	2ME495	1JW495	1JS495	1JE495	1JN495	2JS495	2JW495	2JE495	2JN495
Acidobacteria	*Acidobacteria*	22.73	18.82	26.15	8.92	32.12	25.36	7.80	18.13	15.64	32.44	33.35	41.16	29.27	28.97	28.10	42.83
	Solibacteres	1.41	0.73	0.50	0.33	1.56	1.68	0.86	2.23	1.15	2.51	2.51	2.56	2.22	0.83	3.02	3.75
	Others	1.56	0.05	0.28	0.18	2.37	1.37	0.05	3.45	0.18	0.88	0.65	1.56	1.11	0.13	0.83	4.28
Actinobacteria	*Acidimicrobiia*	0.83	0.28	0.68	0.43	1.23	1.32	0.30	1.80	0.78	0.98	1.43	1.56	0.93	1.09	1.64	1.21
	Actinobacteria	1.11	0.50	1.43	0.48	0.98	2.09	1.87	4.31	2.03	2.54	2.73	1.96	3.53	3.08	7.96	3.12
	Thermoleophilia	1.34	0.43	2.78	0.73	4.99	2.29	1.31	5.30	2.01	4.32	7.52	3.79	3.93	1.92	4.56	4.76
Cyanobacteria		0.13	0.03	0.05	0.08	0.18	0.31	6.13	0.13	0.18	1.36	0.50	0.38	0.83	0.43	0.53	0.20
Proteobacteria	*Alphaproteobacteria*	12.85	18.62	15.37	6.78	30.76	39.22	13.72	38.54	21.53	29.98	35.58	28.03	44.42	44.56	37.02	25.07
	Betaproteobacteria	49.34	52.79	48.81	75.72	15.22	5.67	13.72	9.91	47.38	12.83	3.64	5.95	4.46	8.50	3.65	3.10
	Deltaproteobacteria	0.18	0.13	0.10	0.05	0.28	0.25	0.13	0.33	0.25	0.48	0.53	0.60	0.86	0.68	0.81	0.33
	Gammaproteobacteria	5.82	6.64	1.96	5.73	7.05	14.90	52.72	10.80	6.42	4.67	7.32	7.23	4.46	5.83	5.21	5.09
Bacteroidetes	*Saprospirae*	0.63	0.23	0.63	0.08	0.88	0.64	0.55	0.48	0.75	1.05	1.48	1.26	1.39	1.54	0.81	1.08
	Sphingobacteriia	0.33	0.45	0.08	0.15	0.28	0.31	0.25	0.28	0.38	0.55	0.38	0.50	1.26	0.96	0.83	0.66
	Others	0	0	0.03	0.03	0	0	0	0.03	0.08	0.05	0.05	0	0.08	0.15	0	0
Firmicutes	*Bacilli*	0	0	0	0	0	2.06	0	0	0	0	0	0	0	0.05	0	0
	Clostridia	0	0	0	0	0	0	0	0.05	0	0	0.05	0	0	0	0	0
Other		1.74	0.33	1.18	0.35	2.09	2.54	0.58	4.23	1.25	5.37	2.28	3.47	1.26	1.29	5.04	4.51

Fig. 1. Heatmap of relative abundance of bacterial 16S rRNA gene sequences.

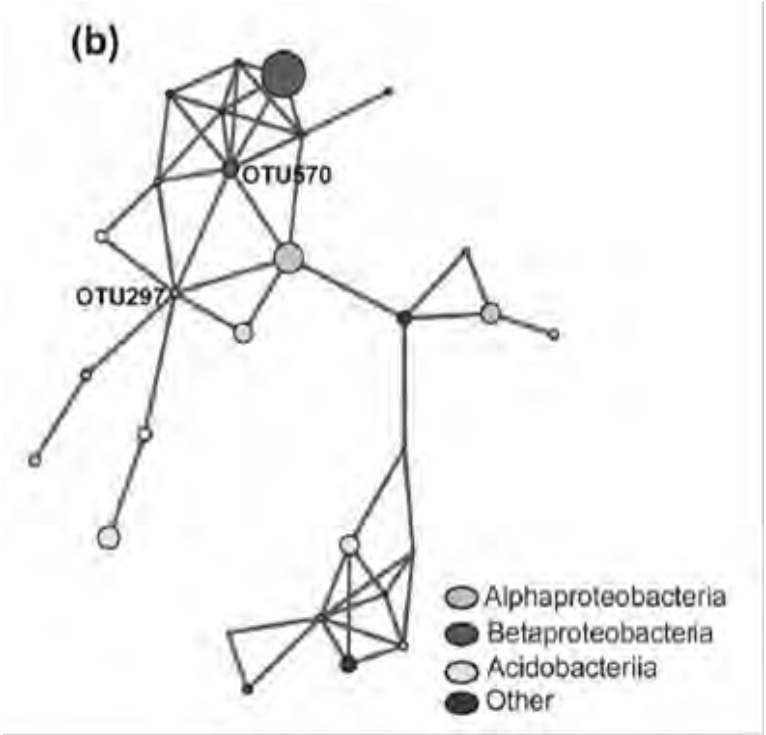

Fig. 2. Community network based on spearman rank correlations between pairs of OTUs. Nodes are sized relative to average abundance of the OTU and coloured according to phylogenetic class. Only OTUs with significant ($p < 0.05$) correlations are shown.

Poster A-07

SPATIAL PATTERNS OF METHANE-CYCLING MICROORGANISMS IN SOILS OF A HIGH-ALPINE ALTITUDINAL GRADIENT

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Methane-cycling microbes play a unique role in the global carbon cycle as both producers (methanogenic archaea) and consumers (methanotrophic bacteria) of the greenhouse gas methane (CH_4) in soils. Traditionally, methanogenic archaea were thought to be restricted to anoxic environments, but nowadays an increasing body of research supports the idea that methanogens can even thrive and be active in aerated uplands including alpine soil, although they are facing harsh environmental conditions (e.g. low temperatures, high UV radiation, poor water availability and nutrient supply) in high-altitudinal habitats.

Our study intended to reveal whether and to which extent microorganisms involved in the global CH_4 cycle are present in high alpine soils, and to assess their spatial distribution along an altitudinal gradient ranging from 2700 to approximately 3500 m above sea level on the south-western slope of Mount Schrankogel, Austrian Central Alps. The three studied vertical zones included (1) the alpine altitudinal belt (2700 to 2900 m), (2) the alpine-nival ecotone (3000 to 3100 m), and (3) the nival altitudinal belt (3200 to ~3500 m). Methanogenic abundances were measured using quantitative real-time PCR (qPCR) targeting the 16S rRNA genes of the following phylogenetic groups: *Methanosarcinales*, *Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*, and *Methanocella*. Methanotrophic bacteria were quantified targeting the functional gene *pmoA*. Methanogenic and methanotrophic potentials were measured by using soils incubated under anaerobic conditions and soils incubated at atmospheric CH_4 concentrations, respectively. Both, CH_4 formation and depletion were monitored via gas chromatography. To connect abundance and metabolic potentials of methane-cyclers to environmental conditions, a range of physicochemical soil properties and soil temperatures were determined.

Methanococcales and *Methanocella* were most abundant among all measured methanogenic groups, while *Methanosarcinales* could not be reliably quantified in any soil along the altitudinal gradient. A clear declining trend along with increasing altitude was observed for the abundance of methanotrophs, *Methanococcales*, *Methanomicrobiales* and *Methanocella*, however not for *Methanobacteriales*. Potential metabolic activities of methanogens and methanotrophs followed a similar trend. Soils sampled at the alpine belt harboured significantly more methanogens and methanotrophs compared to those sampled at the nival belt, while the alpine-nival ecotone represented a transitional area with intermediate abundances. Thus, changes of microbial activities and abundance followed similar patterns to those known for plant coverage. The relationship between abundances and potential microbial activities with mean annual soil temperature was in most cases stronger compared to the relationships with physicochemical soil properties.

Our results show that methane producers and consumers are present in high-altitudinal soils, in spite of harsh environmental conditions. Abundances were clearly declining with increasing altitude and roughly coincided with plant coverage, thus highlighting the importance of vegetation for structuring microbial distributions. Since alpine regions are expected to be among the environments most impacted by climate warming, our results may be useful to predict future changes.

KEYWORDS: ALPINE SOIL; BIOGEOGRAPHY; ALTITUDINAL GRADIENT; METHANOGENS; METHANOTROPHS; VEGETATION

Poster A-08

DIVERSITY AND SUCCESSION OF AUTOTROPHIC MICROBIAL COMMUNITIES IN HIGH-ELEVATION SOILS ALONG DEGLACIATION CHRONOSEQUENCE

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INTRODUCTION

Tibetan Plateau (TP), >4000 m above sea level (a.s.l.), hosts more than 100,000 km^2 of glaciers, which have rapidly been retreated by climate warming (Yao et al. 2012). Glacier retreatment exposes new deglaciated soils, which serve as an ideal natural laboratory to study primary succession of microbial community and soil development. Autotrophic microbes represent pioneering colonizers in deglaciated soils and are key determinants of deglaciated soil development (Schmidt et al. 2008). However, the diversity and structure of autotrophic microbial communities remain largely unexplored in deglaciated soils, particualrly in high-elevation mountains.

METHODS AND MATERIALS

The study site was in the Zhangdang (ZD) glacier in southern TP, with a retreat rate around 10.0 m yr^{-1} during 1970-2007 (Kang *et al.*, 2007). The soils were sampled every 5 m starting from the ZD glacier terminus representing new deglaciated soil to the far side representing aged soils (10 years). A total of 21 soil samples were collected along the deglaciation chronosequence. Soil physicochemical properties were measured by standard methods and soil autotrophic microorganisms carrying key carbon fixation gene, RubisCO (*cbbL* forms IA, IB, IC and ID) were investigated using quantitative PCR and clone library-based sequencing.

RESULTS

Soil total organic matter (TOC) and total nitrogen (TN) inceased with the 10-year chronosequence. The abundance of bacterial 16S rRNA gene and three forms of *cbbL* gene (form IA/B, IC and ID) dramatically increased in half year of deglaciation and then gradually increased with the deglaciation chronosequence, and exhibited possitive correlations with TOC and TN ($P<0.01$). Form IA/B abundance was the highest among three forms of *cbbL* gene. All form IA/B autotrophic microbes were Cyanobacteria, containing Nostocales, Oscillatoriales and Chroococcales, with the average frequency 9.86%, 54.23%, and 35.92%, respectively. Nostocales-like Cyabobacteria were more frequently observed in older soils peaking in 6-years old soil (28.0%). Oscillatoriales-like Cyanobacteria exhibited the highest frequency in the 0.5-year old soil (100%) and the least in 2.5-years old soil (3.57%). The Chroococcales frequency exhibited a substantially temporal variation with a peak in 2.5-years old soil (96.43%). Form IC microbes were Proteobacteria consisting of Actinomycetales, Burkholderiales, Rhodospirillales and Chromatiales and ID sequences were affiliated with Chroococcales, Nostocales, Oscillatoriales, Coscinodiscophyceae and Xanthophyceae. Shannon's diversity and evenness of autotrophic microbial communities gradually increased in young soils (<5 years after glacial recession), and then kept stable.

DISCUSSION

Our data indicates a quick colonization of autotrophic microbes in high-elevation deglaciated soils, and their structure showed a substantial shift along the 10-year deglaciation chronosequence. The possitive correlations of soil TOC and TN contents with bacterial and autotrophic microbial abundance suggest that the soil nutrients were originated from autotrophic microbes. The autotrophic microbial abundance and their diversity were structured by deglaciated soil age, but not by ancient nutrients.

KEYWORDS: TIBETAN PLATEAU, DEGLACIATED SOILS, AUTORTROPH, MICROBIAL COMMUNITY, GLACIER, *cbbL*

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Poster A-09

SOM MINERALIZATION SENSITIVITY TO TEMPERATURE AND O_2 AVAILABILITY IN DEEP PEAT PROFILES INCLUDING PERMAFROST INTERFACE

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Globally significant pool of soil organic carbon (SOC) (Tarnocai et al. 2009) is stored in arctic peatlands where extensive permafrost prevents the decomposition of old soil organic matter (SOM). Vulnerability of ancient organic depositions in changing environment becomes a considerable issue in future climate models. Palsa mires, a typical cryogenic peatland type in subarctic tundra, are not only an important SOC pool but also have been reported as a source of nitrous oxide (N_2O) (Marushchak et al. 2011). Microbial SOM mineralization and its sensitivity to changing environmental conditions are crucial to understand future C losses and greenhouse gas (GHG) fluxes in this abundant landform of subarctic region.

A preceding ex situ thawing experiment with intact palsa mire soil cores was performed in order to mimic impact of climate change on deep soil profiles with a permafrost layer. Surface GHG emissions were monitored during the gradual permafrost thawing and evaluated in relation to availability of C and N forms. Our succeeding study was launched afterwards with a purpose to determine potential SOM mineralization in separate layers of the deep soil cores. First, we aim to define a response of C losses and GHG exchange rates to temperature and aerobic/anaerobic conditions in different peat layers. Secondly, we seek for relations among SOM mineralization, nutrient availability and parameters of indigenous microbial community. Finally, we attempt to link the potential SOM mineralization of the peat layers with surface GHG fluxes from the former study.

We separated five peat soil cores into five layers (0~20, 20~40, 40~60 cm, permafrost interface +/- 5 cm, permafrost layer). Homogenized soil samples were incubated in a factorial set-up of three temperatures (4, 10, and 16 °C) under aerobic as well as anaerobic conditions. At the beginning and the end of the total 5.5-months incubation period, we determined C and N availability, C-substrate quality, microbial biomass, potential activities of extracellular enzymes, target genes for C and N mineralization processes and composition of microbial community (fungi/bacteria ratio). Heterotrophic respiration (CO_2), methane (CH_4) and nitrous oxide (N_2O) emissions were monitored weekly at the initial phase and biweekly later during the incubation. All data are currently being processed.

The present study addresses the essential question to which extent deeper soil horizons of subarctic organic depositions contribute to the total soil GHG fluxes, and whether the nutrient availability, SOM quality, microbial community and environmental factors (i.e. temperature, O_2 availability) constrain the SOM mineralization.

KEYWORDS: SOM MINERALIZATION, PERMAFROST THAW, PALSA MIRE, GHG

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Poster A-11

ROLE OF SOIL ALGAE AND CYANOBACTERIA IN COLONIZATION AND SUCCESSION ON DEGLACIATED SOILS IN HIGH ARCTIC (SVALBARD) AND ALPINE/SUBARCTIC REGIONS (SCANDINAVIA)

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Forelands of glaciers receding due to global warming offer unique possibilities for research on primary succession and ecosystem development. Although photoautotrophic microorganisms are generally thought to be important colonizers of barren areas, relatively little is known about the role of particular groups in deglaciated areas of Northern Europe. The main goal of this study was to compare the development of algal and cyanobacterial communities along glacier retreat in High Arctic (Svalbard) and in subarctic/alpine regions in Scandinavia. To understand the role of particular groups/species of soil algae and cyanobacteria as well as main factors affecting development of algal and cyanobacterial communities during succession we selected forelands differing in climatic conditions, bedrock, altitude, slope, exposure, etc. Five glacier forefields were sampled, three -Hørbyebreen, Ferdinandbreen, Ragnarbreen- in the vicinity of Petuniabukta Bay (Billefjorden, Central Svalbard), two - Storglaciären (Tarfala, Sweden) and Midtdalsbreen (Finse, Norway) in Scandinavia. Five/six successional stages were established along chronosequences from recently deglaciated sites at the glacier fronts (initial successional stage) to old successional stages developed since the end of LIA (climax). Species composition, dominant species/groups and abundance of algae were studied by using direct epifluorescence microscopy and cultivation methods in soil samples collected aseptically from surface soil layers. Airborne propagules were caught on Petri dishes with mineral agarized media exposed in initial and early successional stages.

Similar successional trend in algal community development was observed in all studied forelands. Six to 14 algal species were isolated already in the initial, barren successional stages, small diatoms and xanthophytes (*Xanthonema, Heterococcus*) usually prevailed. Both species numbers and abundances rapidly increased with increasing succession age reaching maximal values of more than 30 species and abundances typical for developed temperate soils in younger (in Scandinavia) or older (in Svalbard) succession stages and showing decreasing tendency in oldest stages or climax. Class Trebouxiophyceae was the most diverse algal group found in studied forelands, species of *Pseudococomyxa*, *Elliptochloris, Chloroidium, Stichococcus* belonged to the most frequent and often dominant species. These species were also frequently isolated from air.

The development of cyanobacterial communities was driven by soil pH. Cyanobacterial communities played an important role in all successional stages along all chronosequences in Svalbard characterized by alkaline to neutral soil pH. In Scandinavia, cyanobacterial communities occurred only in younger successional stages, and disappeared with decreasing soil pH from neutral to highly acid values, typically in older successional stages and climax. Oscillatoriaceae dominated in all studied forefields, different *Leptolyngbya* species, colonizing already initial barren soils, were most important. Species of Nostocales and Chroococcales and other Oscillatoriales occurred in early/young successional stages in Scandinavia, and in young/older stages in Svalbard.

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KEY WORDS: COMMUNITY DEVELOPMENT, SOIL pH, AIRBORNE PROPAGULES, CHRONOSEQUENCES, GLACIER FORELANDS

Poster A-12

EFFECT OF ALTITUDE AND SEASON ON MICROBIAL FUNCTIONALITY, COMMUNITY STRUCTURE AND ABUNDANCE IN ALPINE FOREST SOILS

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Altitudinally and seasonally defined climatic conditions influence soil microbial communities and their metabolic activities to a significant extent. However, knowledge on the effect of changed climate conditions on soil microorganisms in forest ecosystems is still insufficient.
We characterized four forest sites located in South Tyrol, in the Italian Alps, along an altitude gradient (545-2000 m), including submontane, montane, subalpine and alpine vegetation levels, to evaluate the effect of altitude and season (spring and autumn) on soil microbial communities. Two of the forest sites are part of the long-term monitoring study "International Cooperative Programme on Assessment and Integrated Monitoring of Air Pollution Effects on Forests (ICP-IM)". The characterization of soil samples included: soil microclimate, physical and chemical properties, the determination of the structure and abundance of microbial communities by phospholipid fatty acid (PLFA) analysis and the determination of community-level physiological profiles (CLPP), using the Biolog EcoPlate system, basal respiration as well as key enzyme activities involved in C, N, P and S cycles and in litter degradation. The results showed that soil organic matter and nutrient content increased with altitude in both seasons, which could be related to the higher total microbial abundance also found at these sites. Respiration and enzymatic activities reached the highest values in soils from subalpine and alpine sites. Independent of altitude, significant differences between seasons were not detected. Multivariate analysis of all the data analysed in the present study allowed us to conclude that the most important factor determining microbial functionality, community structure and abundance was the altitude and not the season.

KEYWORDS: FOREST SOILS, ENZYMES, RESPIRATION, PLFA, ALPINE

Poster A-13

SHIFTS IN MICROBIAL COMMUNITY STRUCTURE IN A GLACIER FOREFIELD (STYGGEDALSBREEN, CENTRAL NORWAY)

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Styggedalsbreen, a small sub-arctic valley glacier located in western Jotunheimen (central Norway), has retreated approximately 800 m since the Little Ice Age (c. 1750 AD) and its forefield has become successively exposed to environmental changes as a consequence of increasing temperatures. Here, we present a study with the aim to obtain new insights into the structure and diversity of microbial communities along a proglacial longitudinal transect (a chronosequence). The results show how different abiotic factors develop with exposure time and we assess their impact on the microbial communities. Soils from six different stages along the chronosequence were collected and several analyses were performed. Samples were analysed for organic carbon (OC) and total nitrogen (TN) with a CHN analyser, while pH (w/w) and 1M KCl (w/w) were measured to determine the actual and potential acidities of the soil samples. To estimate the microbial diversity, 16S rDNA copy numbers were assayed. Two different sets of primer-pairs were used to compare the abundance of both Bacteria and Archaea. "Next-generation sequencing technology" was applied to study the microbial community composition and distribution along each stage. The effect of temperature was observed by incubating samples to 5°C and 22°C during one week. Results from Illumina sequencing showed a shift in the microbial community composition along the proglacial transect, when the incubation temperature was low (5°C) whereas at high temperature (22°C) the community structure was stable. Analyses of abiotic factors revealed an increase of OC as a function of soil age (from 0.39 mg/g to 53.06 mg/g) due to the increment of microbial biomass and its activity. The TN values were low but they increased with soil age along the proglacial transect (only up to 2.9 mg/g). However, in some samples the TN values were below the detection limit. The pH values changed towards more acidic (7.89 to 4.74) with exposure time due to soil development (pedogenesis). Using 16S rDNA as target for amplification, the copy number values were found to be low for members from both Bacteria and Archaea domains. However, in both cases the copy number values increased with soil age along the proglacial transect, where the increase was more marked for members from Bacteria compared to members from Archaea.

Members from *Proteobacteria* (especially *β-Proteobacteria*), *Bacteroidetes*, *Chloroflexi* and *Euryacheota* phyla among others dominated the recently deglaciated areas. Therefore, methane generation along the first stages of the chronosequence would be expected, as these newly exposed areas are adequate ecosystems for the anaerobic conditions required by members from *Euryarchaeota* (such as methanogens) and also for members from *Taumarchaeota*, which can catalyse the ammonia oxidation metabolism during the last stages of the chronosequence. Moreover, other microbial processes such as carbon dioxide production, sulphur oxidation and methane oxidation are predicted based on the presence of members from *Oxalobacteraceae*, *Comamonadaceae*, and *Methylophilaceae* families (*β-Proteobacteria*). Very few nitrogen-fixing organisms were identified, probably due to the non-enabling (physic-chemical) environment and the low TN concentration values along the chronosequence. The results from Styggedalsbreen forefield add new insights into our current understanding of changes in proglacial microbial ecosystems, emphasizing the consequences of the global warming in this environment.

KEYWORDS: CHRONOSEQUENCE, GLACIER FOREFIELD, STYGGEDALSBREEN, MICROBIAL COMMUNITY, *β-PROTEOBACTERIA*.

Poster A-14

COMPOSITIONAL SHIFTS IN ECTOMYCORRHIZAL FUNGAL COMMUNITY IN RESPONSE TO LONG-TERM SNOW DEPTH INCREASE

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Climate warming is inducing changes throughout the Arctic altering land, sea and atmospheric processes and interactions. Many of these changes could potentially amplify climate warming via positive feedbacks. Tundra responses to changes in climate include aboveground changes, such as reduction in albedo associated with shrub expansion and tree line advance, and shifts in ecosystem C cycling resulting from greater C fixation. Importantly, the net effects of these climate-induced changes are unresolved, in part because our understanding of belowground responses to changes in winter conditions are limited. Here we focus on the effects of increased winter precipitation, and as a consequence, increased winter soil temperatures during the cold season, in arctic ectomycorrhizal (ECM) fungal communities in dry and moist tussock tundra. For that purpose we analyzed DNA data generated by Ion Torrent sequencing of soil samples taken at a long-term (18-year) snow fence experiment set up in Toolik Lake, Alaska. The results pointed to a significant effect of the snow depth in the arctic ECM fungal community. In the dry tundra, both the community richness and composition were significantly altered. There was a particular and sharp decrease in richness in *Tommentella*, *Inocybe* and taxa with contact, short distance and medium distance smooth hyphal exploration types. On the other hand, *Cortinarius* richness did not change resulting in a proportional increase of taxa with medium distance fringe hyphal exploration type that has been argued to be able to scavenge the soil for recalcitrant N forms. On the moist tundra, only the community composition changed, richness did not, and there were strong OTU-specific responses to the altered conditions. Our findings indicate that ECM responses to deeper snow in winter are tundra-type dependent. The shifts in the ECM composition may accelerate tundra plant's ability to acquire growth-limiting resources and that the coupled changes in above- and belowground processes point to a synergistic relationships between vegetation and ECM fungi.

KEY WORDS: CLIMATE CHANGES, FUNGAL ECOLOGY, ARCTIC ECOLOGY, TOOLIK LAKE, ITEX

Poster A-15

COMPLEX AND VARYING LICHEN MICROBIOMES ACCORDING TO THE VERTICAL POSITIONS OF THALLI IN *Cladonia gracilis* FROM KING GEORGE ISLAND, ANTARCTICA

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Lichens are symbiotic organisms that are majorly composed of lichenized fungi (mycobiont) and green algae/or cyanobacteria (photobiont). However, lichen thalli also contain highly diverse microbes such as bacteria, archaea and microfungi (microbionts) as well as mycobiont and photobiont. Although research interests on ecological functions of microionts have been raised greatly, very little knowledge on this topic has been gained, especially on microbionts of lichens from extreme environments. Microbial communities are usually affected by subtle changes of environments and different parts of lichen colonies, *Cladonia gracilis*, which forms lichen colonies composed of many slender individual thalli, may provide different micro-environments to the microbial communities of *Cladoia gracilis* from King George Island, Antarctica. To reveal the microbial communities of different parts of the lichenized fungi, we sectioned thalli from middle, intermediate, and marginal positions of colonies to apical, middle and basal parts. Since apical parts are usually more exposed to sun light and wind, and has lower humidity compared to basal parts, we considered this sectioning to represent different conditions. Bacterial 16S rRNA gene and eukaryotic nuclear large subunit rRNA gene (LSU) were analyzed by 454 pyrosequencing method. *Alphaproteobacteria* and *Acidobacteria* were the major bacterial phyla in all parts of the thalli with varying abundance. Apical parts of thallii contained relatively simple microbial communities that were majorly composed of Acetobacteriaceae and *Acidobacteriaceae*. Basal parts contained much more complex microbial communities with diverse phyla and enriched with *Actinobacteria*. LSU sequences were mostly composed of that of *Cladonia gracilis*, but also contained diverse fungal species including *Lecanoromycetes*, *Leotiomycetes* and *Dithideomycetes* with varying abundance depending on the vertical positions. High abundance of *Ochrolechiaceae* and *Stereocaulaceae* and diverse *Cladonia* genotypes in *Cladonia gracilis* thalli observed in this study may imply complicated life style of potentially lichenized fungi and complex composition of lichen thalli. Diverse algal species and different composition according to the vertical position provide insights on fungal selectivity to algal genotypes depending on the micro-environmental conditions.

KEYWORDS: LICHEN; *Cladonia gracilis* COMPLEX; MICROBIOME

Poster A-16

IMPACT OF HUMAN PRESENCE AND ACTIVITY ON ECOLOGY AND ADAPTATION OF AN ANTARCTIC PSYCHROPHILIC BACTERIA COMMUNITIES

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INTRODUCTION

Antarctica, due to extremely low temperatures, hardly available organic matter and limited access to sunlight, is one of the least friendly regions on Earth where life evolved. However, bacteria, masters of adaptation to extreme conditions, can dwell even there.

Antarctic harsh climatic conditions have made it one of the least altered by human regions on Earth and hence it is an ideal place to study the evolution of microorganisms, especially bacteria. In this work we have performed the comparative analyses of microbial communities of psychrophilic bacteria inhabiting two Antarctic ecological niches: (i) Jardine Peak, the primal area of rich brown soil, and (ii) the soil at the vicinity of an industrial building of the Arctowski Polish Antarctic Station (the environment interfered by human activity). We focused on comparing the diversity of cultivable microorganisms, their physiological characteristics and the identification of mobile genetic information carried by identified bacteria.

METHODS AND MATERIALS

We used common microbiological and molecular methods to identify bacteria, analyse their physiology (temperature, pH and heavy metal ions tolerance) and isolate plasmids. *In silico* methods for plasmid annotation (similarity searches, multiple sequence alignments, identification of protein conserved domains) were applied.

RESULTS

We have obtained 149 strains from two sites located on King George Island (Antarctica): Jardine Peak, which are relatively fertile soil (59 strains) and soil sample taken near the industrial building (pumping fuel), which is part of the technical infrastructure of Arctowski Polish Antarctic Station (90 strains). All of bacterial strains were assigned to proper taxonomic groups.

We have observed a much greater bacterial diversity in soil collected near Arctowski Station (12 species, including *Arthrobacter*, *Psychrobacter*, *Pseudomonas*, *Pedobacter*, etc.). In contrast, in the soil sample from Jardine Peak 59 gram-negative bacterial strains belonging to 4 species (*Pseudomonas*, *Polaromonas*, *Flavobacterium* and single strain of *Janthinobacterium*) were found.

Physiological analyses showed that the strains isolated near the Arctowski Station have a higher pH optimum, much higher tolerance to salinity and can tolerate higher concentrations of heavy metal ions (especially As, Zn, Cu, Ni and Cr) comparing with the Jardine Peak isolates.

The plasmid analysis of the bacterial strains showed that bacteria inhabiting the neighborhood of industrial buildings usually contained at least one plasmid (69 % of analysed strains). Interestingly 36% of analysed strains were multi-replicon isolates. In contrast, among bacteria isolated from Jardine Peak plasmid-less (58%) and single-plasmid cells (25%) dominated. To date we successfully sequenced and annotate 85 plasmids from 38 strains but our studies are still in progress. We have also evaluated the possible role of identified plasmids in bacterial adaptation and ecology.

DISCUSSION

The obtained data showed that the human presence strongly influences Antarctic bacteria communities. Human activities brings the selection pressure on bacteria and accelerates their evolution. Bacteria have to adapt rapidly to changing environmental conditions. This can be enhanced by the plasmids, which are the major players in horizontal gene transfer. Plasmids may encode various phenotypic modules enhancing bacterial fitness, therefore their analysis is extremely important to understand the biology and diversity of extremophilic bacteria.

KEYWORDS: PSYCHROPHILIC BACTERIA, ADAPTATION, HEAVY METAL RESISTANCE, PLASMID

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Poster A-17

CLIMATE FEEDBACK OF ARCTIC ECOSYSTEMS: WARMING ENHANCES NUTRIENT TURNOVER AND ALTERS CARBON AND NITROGEN FLUX DYNAMICS IN SUBARCTIC TUNDRA

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Arctic peatlands are known to play a major role in the global carbon cycle, since they store large parts of the earth's soil carbon. They are considered to be a sink for carbon dioxide (CO_2) und a small source of methane (CH_4). Recent findings suggest that those arctic regions can further be a relevant source of nitrous oxide (N_2O), with N_2O hotspots found in areas of naturally bare peat without vegetation (Repo et al. 2009). N_2O fluxes in these regions were neglected in previous studies as they were believed to be insignificant in such nutrient poor ecosystems. Predicted climatic changes will have an uncertain impact on the greenhouse gas (GHG) balance of these highly sensitive ecosystems. However, the response of ecosystem processes and related GHG fluxes may differ largely across the landscape depending on soil type, vegetation cover, and moisture conditions.

In this study we investigate how temperature increase reflects on GHG fluxes (CO_2, CH_4 and N_2O) from various tundra surfaces in the Russian Arctic (67°03' N 62°55' E). These surfaces include raised peat plateau complexes, mineral tundra soils as well as bare peat surfaces. Predicted temperature increase is simulated by means of open top chambers (OTCs), which are placed on different soil types for the whole snow-free period. GHG fluxes and soil profile concentrations of CO_2, CH_4 and N_2O as well as nutrient concentrations are linked to major underlying biogeochemical processes.

Our results show that even gentle warming (0.5-2°C) of the upper soil layer significantly impacts on carbon and nitrogen dynamics in arctic permafrost soils. We found increased dissolved organic carbon concentrations in the uppermost soil layer as well as increased ecosystem respiration rates from the warmed surfaces, while at the same time photosynthesis rates were lowered, significantly decreasing the sink function for CO_2 within all measured surface types. CH_4 emissions, generally negligible from dry permafrost peatlands, increased four-fold within the bare peat surfaces, with maximum rates of up to 8.9 mg CH_4 m^{-2} d^{-1}. Warming also altered processes related to N_2O emissions. Soil profile concentrations of inorganic nitrogen (nitrate, ammonium) were found to be lower within the warmed soils, indicating a higher turnover of soil nutrients and changes in the activity of the microbial biomass in respect to warming. N_2O fluxes were controlled mainly by nitrate concentrations within the soil and ^{15}N values suggest that denitrification is the main process resulting in N_2O emissions.

A changing climate will alter uptake and emission behaviour of pristine arctic peatlands, as both the carbon and nitrogen cycle are highly sensitive to warming. Interactions between warming, small-scale hydrological conditions as well as associated changes in vegetation compositions will reduce the sink function of subarctic tundra and impact on the GHG balance of these ecosystems.

KEYWORDS: CLIMATE CHANGE, ARCTIC, CARBON, NITROGEN, PERMAFROST

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Poster A-18

MICROBIAL COMMUNITY RESPONSES TO FUTURE CLIMATE CHANGE AND SEASONAL VARIATION IN ARCTIC TUNDRA SOILS

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Soil microbial communities represent key players in the decomposition of soil organic matter. Future climate changes are expected to have the most pronounced effects in Northern latitudes and sensitive arctic ecosystems will be therefore subjected to dramatic shifts. The response of belowground organisms to these changes and thus their altered impact on nutrient cycling and green house gas production has received little attention, limiting our ability to predict future ecosystem functioning.

The aim of this work was to compare soil microbial communities from control and climate-manipulated plots with respect to seasonally fluctuating environmental factors and ecosystem processes. The study is part of snow cover manipulation experiment that was established on Disko Island, West Greenland in summers 2012 and 2013 at dry and wet tundra, respectively. The experiment simulates the impact of deeper snow cover on tundra ecosystem as increased winter precipitation is expected in most Arctic regions in future decades. Topsoil samples from manipulated and control plots were collected in June, July, September and October of 2014. To assess the diversity of bacterial and fungal communities, the method of amplicon sequencing of 16S rDNA and ITS region on Illumina MiSeq platform was used.

Analysis of sequences revealed distinct differences in diversity of microbial communities from dry and wet tundra soils. Richness of bacterial community in wet tundra was significantly higher compared to dry area, whereas fungal community showed opposite pattern. Fungal community richness was more affected by the manipulation treatment and by seasonal effects than bacterial community. Deeper snow cover led to shifts in microbial community composition on both studied areas. The results of the study show relatively fast response of soil microbes to the climate manipulation treatment indicating their sensitivity to future climate changes.

KEYWORDS: SOIL MICROBIAL COMMUNITIES, SNOW COVER MANIPULATION, CLIMATE CHANGE, ARCTIC TUNDRA, SEASONALITY

Session B
Microbial diversity and evolution

Keynote lecture KN-B

SO WHAT IS IN THE ATMOSPHERE - THE LAST PIECE OF THE JIGSAW?

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Since the turn of the last century, scientists have been collecting data on the biodiversity and evolutionary relationships of microorganisms in the Polar and Alpine regions. However, we are still far from a comprehensive understanding of the total level of microbial diversity in many environments. For a long time it was considered that many of the microorganisms found were unique and unusual due to the perceived extreme nature of the environment. As time has passed and with the advent of new technology, we are starting to gain a much clearer understanding of how true this assertion is, for example, the growing evidence of microbial endemism. Indeed, patterns are starting to emerge, such as the cold-adapted genera, but we are still far from a full understanding and it is probably still true to say that the harder we look, the more we find. Much of the effort in understanding biodiversity and biogeography in the polar and alpine regions, to date, has overlooked the key consideration of stability. Potential colonists arrive continually from the atmosphere in both precipitation and as wind-blown debris, yet next to nothing is known about the fate of new colonists or the rate of such changes. Knowing this rate is fundamentally important in understanding whether or not any particular environment can cope with induced change. In this session, we will review the status of our understanding, examine the large questions which still remain and suggest approaches by which our understanding may be taken forward.

Lecture B-01

VARIETY AND DIVERSITY OF REPRESENTATIVES OF 'CANDIDATE' PHYLA IN COLD SEEPS FROM SAYAN MOUNTAINS (SIBERIA, RUSSIA)

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Cultivation independent molecular surveys give us unique information about the large majority of these organisms. Diversity and structure of microbial communities based on conserved marker genes (SSU rRNA) or through shotgun sequencing (metagenomics) have been intensively studied (Amann et al. 1995, Rajendhran et Gunasekaran 2011, Gilbert et Dupont 2011). An increasing number of microbial 'species' revealed more then 100 phyla within the bacterial and archaeal domains, which have no cultivated representatives (so-called 'candidate' phyla).

Eastern Sayan mountain range has complex orographic structure and is characterized by a combination of high ridges and deep valleys with patches of plateaus and plateaus with absolute evaluation of watershed consists of 1900-2700 m. Network of surface waters is well-developed in Eastern Sayan and represented by rivers, lakes, bogs and glaciers. The object of our study was cold seeps, located on the shore of Lake Arshantai-Nur in the headwaters of the river Hoyto-Gol.

Water samples were collected from the three seeps (S7-2, S7-4, S7-8) in August, 2011 for complex analysis. Physico-chemical characteristics of water were determined using a field laboratory Multi-340i (WTW, Germany). The main ion composition and elements were studied by the methods of collometry combined with ICP-MS.

There were no biomats observed in all seeps, but their sediments varied significantly. Seep S7-2 was characterized by clay-like sediments and milky suspended matter in the water. Seep S7-4 had highly transparent water and small jack-stones in soft sediments. Ocherous suspended matter was observed in the seep S7-8 supposed high iron content in the sediments. Among physicochemical characteristics of waters from seeps only mineralization (55.3 mg/L in S7-4 and 89.6-105.0 mg/L in S7-8, S7-2) and dissolved oxygen (1.78 mg/L in S7-2 and 10.18-12.18 in S7-4, S7-8) differed significantly, water temperature and the pH values were relatively similar 0.9°C and 7.24-7.84, respectively. According to the main ions composition waters from seeps S7-2 and S7-4 varied significantly from seep S7-8: concentration of main ions, as well as Si was 3 to 5 times higher. The highest concentrations of Fe and Mn were detected in seep S7-8 and they were 2.5 and 1.4 mg/L, respectively. The seep S7-2 with milky suspension was characterized by high content of Al, Ti and As (70.85, 3.80 and 1.67 µg/L, respectively). The highest concentrations of Sr, Li, and U were detected in the transparent seep S7-4 and they were 216.44, 8.16 and 3.05 µg/L, respectively.

Metagenomic analysis of 16S rRNA gene fragments showed a high bacterial diversity in waters of the seeps and revealed five major phyla of Eubacteria: Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria and Verrucomicrobia with dominance of Bacteroidetes in S7-4, Cyanobacteria in S7-2, and Verrucomicrobia in S7-8. Additionally, Firmicutes, Chlamydiae and Acidobacteria were detected. Notably, the fraction of unclassified bacteria was very high and varied insignificantly (12.8-14.8%). Minor bacterial phyla were very diverse and presented by 12 different eubacteria phyla, as well as candidate phyla Parcubacteria, Latescibacteria, Microgenomates, Omnitrophica, Candidatus Saccharibacteria, candidate divisions WPS-2, WPS-1, ZB3, SR1. Recent research on single-cell genomic allowed to sequence whole genome of uncultivated bacteria of phyla Parcubacteria, Microgenomates, Latescibacteria, Omnitrophica from freshwater Sakinaw Lake and proposed their metabolic activity (Rinke et al., 2013). It was shown that genomes of these organisms possess genes for the degradation of amino acids and sugars, pointing to a heterotrophic lifestyle (Rinke et al., 2013). No evidence for the ability to perform a more complete set of cellular respiration processes were found in the Parcubacteria, Microgenomates and Latescibacteria (Rinke et al., 2013). Thus we described new habitats comprising bacterial populations belonging to high diversity of candidate phyla.

KEYWORDS: COLD SEEPS, WATER CHEMISTRY, DIVERSITY, SAYAN MOUNTAINS, METAGENOMIC ANALYSIS

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Lecture B-02

UNRAVELING THE UNKNOWN MICROBIAL DIVERSITY HIDDEN IN ALPINE PERMAFROST

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Permafrost constitutes around one fourth of the terrestrial ecosystems, representing a unique ecological niche for psychrophilic and psychrotolerant microorganisms. There is an increasing concern that thawing of permafrost with global warming will promote microbial activity and release massive amounts of potentially labile carbon with direct feedback on the greenhouse gas budget. Less attention, however, has been given to the possibility that thawing of permafrost will unlock unknown and potentially novel microbial diversity including pathogenic or other microorganisms of environmental concern. Therefore, gaining a better understanding of the microbial diversity hidden in permafrost soils is interesting from both an ecological and evolutionary perspective.

We, therefore, aimed to unravel the structure and diversity of the prokaryotic and eukaryotic microbiome in old permafrost soils and its overlying active soil layer by using massively parallel sequencing of ribosomal marker genes on the Illumina MiSeq platform. For this purpose, we collected samples from two high-alpine sites in Switzerland for which we could date back the permafrost to be approximately 5,000 and 12,000 years old, respectively, using ^{14}C radiocarbon dating. The microbiological data were compared to the simultaneously collect edaphic physico-chemical background of the samples, including soil texture, carbon and nitrogen content, nutrients and pH.

The sequencing yielded over 5 million high-quality ribosomal reads of prokaryotic and eukaryotic domains including bacteria, archaea, fungi, and protists. The permafrost samples harboured a microbiome that was highly distinct from communities in the overlying active soil layer as well as from communities found in deeper, south-exposed and thus non-frozen soil strata at the same site. We found an unexpectedly high microbial diversity in the permafrost compartment with a large fraction of the diversity being phylogenetically distinct from anything known. Indicator species analysis coupled to the construction of phylogenetic association networks allowed us to disentangle the unknown microbial diversity, laying the foundation for upcoming culture-based physiological typing of potentially novel taxa locked up in alpine permafrost. These unique data yielded an unprecedented view on microbial life in permafrost soils and offers a better understanding of the microbial impact on the environment under future climate scenarios.

KEYWORDS: UNKNOWN MICROBIAL DIVERSITY, PERMAFROST, NEXT-GENERATION SEQUENCING, PROKARYOTES AND EUKARYOTES

Lecture B-03

ARCTIC SNOWPACK-SOIL INTERFACE – STRICT BOUNDARY OR ECOSYSTEM TRADING ZONE?

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Microbial diversity in cold soils is influenced by temporal and spatial snow cover dynamics, but the processes mediating this influence remain unclear. We hypothesized that limited nutrients such as nitrogen control microbial communities in these cold soils. Microbial communities in an arctic soil as well as in the overlying snowpack were investigated over six different time points at six replicate field sites throughout one year in Ny-Alesund, Svalbard. In order to examine potential interactions between snow and soil communities, three soil plots were covered with a gas-tight membrane before the snow began to accumulate and three remained uncovered. Samples collected from the plots give information about the interaction of communities via gaseous products derived from nutrient cycling processes. Quantitative PCR to determine marker gene copy numbers of nutrient cycling processes and RISA analysis of fungal and bacterial communities served as methods to test our hypotheses. Based on our results, both fungal and bacterial communities showed shifts in community structure throughout the year, although different patterns were observed. Q-PCR results of ribosomal marker genes showed a change in abundance for fungi throughout the year, which was not found for bacterial communities. Copy numbers of N cycling genes demonstrated changes in abundance for ammonia oxidizing archaea. Our results show that microbial communities in both the snow and the soil are dynamic and that they are correlated to nutrient cycles in Arctic ecosystems.

KEYWORDS: NITROGEN CYCLE, BACTERIAL COMMUNITY, FUNGAL COMMUNITY, SOIL, SNOWPACK

Lecture B-04

DRAFT GENOME OF MEMBERS OF THE OP9 LINEAGE BY SINGLE CELLS SORTED FROM A MARINE SEDIMENT OF THE ROSS SEA, ANTARCTICA

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Members of candidate phylum OP9 are found in geothermal systems, petroleum reservoirs, anaerobic digesters, wastewater treatment facilities, and marine sediments. Yet, little information regarding their metabolic capabilities and ecological role within such habitats is currently available due to the lack of cultured isolates. In this study, 8 single-cell genomes (SCGs) of OP9, which was predominant composing 26.7% in the marine sediments of the Ross Sea, Antarctica was obtained through single-cell sequencing. Eight SCGs showed high 16S rRNA gene identity (>99.3%) each other while they had low 16S rRNA gene similarities (<81%) with those of OP9 genomes obtained from hot spring sediments. The average nucleotide identity (ANI) values among 8 SCGs ranged from 97.7-99.9%, greater than the genomic 95% ANI empirically determined to delineate species. Thus, we combined the data sets to construct a composite SCG (cSCG) assembly. The cSCG assembly result had a total size of 2,846,496 bps containing 2,418 contigs (N50 contig size, 4,011 bps and Max contig size, 42,489 bps), and the G+C contents are 32%. Approximately 85.1% of the draft genome sequence was predicted as protein-coding regions (4,401 genes) and the average of gene length was 550 bps. As a first study on the OP9 genome from Antarctic marine sediment, more detailed analyses will provide the glimpse into the lifestyle of a member of widely distributed, yet poorly understood bacterial candidate division OP9.

KEYWORDS: SINGLE CELL GENOMICS, OP9, NOVEL PHYLUM

Lecture B-05

METHANE TRANSFORMATIONS IN ARCTIC AND ANTARCTIC ICE-COVERED LAKES

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A key feature of ice-covered environments is that they typically receive little to no solar energy. Consequently, organisms inhabiting these environments must rely on chemical energy to assimilate either carbon dioxide or organic molecules to support their metabolism. Methane can be utilized by certain bacteria as both a carbon and energy source. Isotopic data from thermokarst lakes in northern Alaska and permanently ice-covered lakes in the McMurdo Dry Valleys, Antarctica show that methane is derived from both biogenic and thermogenic sources. Thermogenic sources of methane in the thermokarst lakes of the north slope of Alaska yield supersaturated water columns during winter ice cover that support active populations of methanotrophs during the polar night. Methane in the permanently ice-covered lakes of the McMurdo Dry Valleys, Antarctica varies widely in concentration and is produced either by contemporary methanogenesis or is a relic from subglacial flow. Rate measurements revealed that microbial methane oxidation occurs beneath the ice in both the arctic and Antarctic lakes and can be a significant sink at methane before it reaches the atmosphere. Knowledge of methane transformations in ice-covered polar lakes on our planet, together with what we know about the geochemistry of icy worlds beyond earth such as Europa and Enceladus, provide an important analog to assess the metabolic diversity that may exist in extraterrestrial systems.

KEYWORDS: ARCTIC, ANTARCTICA, METHANE, ASTROBIOLOGY

Lecture B-06

IT PAYS TO BE A WINNER: VIRAL CONTROL OF THE BACTERIAL COMMUNITY OF A HIGH ARCTIC GLACIER SURFACE

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Recent work demonstrates that glacial ice surfaces comprise seasonally-evolving three-dimensional photic zones which accumulate microbial biomass and potentiate positive feedbacks in ice melt (Irvine-Fynn and Edwards 2014). Viruses are abundant in glacial systems and high frequencies of visibly-infected cells and very low burst sizes (Säwström et al. 2007) have been found in Arctic cryoconite hole supernatants. Therefore, viral shunting of the microbial loop is probably important in supraglacial carbon cycling (Säwström et al. 2007). Here, the extent and sensitivity of top-down viral control of bacterial communities of supraglacial stream meltwater of Midtre Lovénbreen was investigated.

Firstly, viral decay rates in supraglacial meltwater were used to estimate the viral controls upon bacterial production in the glacial photic zone. We found a potent viral shunt of 35% of bacterial production via viral lysis to dissolved organic matter, which will truncate the flow of carbon to higher trophic levels and modulate supraglacial bacterial growth efficiencies. The virus particles were very stable in supraglacial meltwater, with a predicted half-life of 39 days. Thus, viral control in the glacial photic zone is likely to be an important influence on supraglacial dissolved organic carbon and virus export.

Secondly, the effect of soluble resource availability on virus–bacterium interactions and consequently bacterial abundance, productivity and diversity within the weathering crust was determined by nutrient amendment in experimental microcosms. We found that photic zone bacterial communities can sequester resources to escape viral control, as evidenced by a marked decline in virus-to-bacterium ratio concomitant with increased bacterial productivity and numbers.

Thirdly, V1-V3 16S rRNA gene pyrosequencing revealed changes in bacterial communities in the microcosm experiments. We found that a few bacterial taxa were consistently dominant, with the most dominant OTU affiliated to *Janthinobacterium* sp. (≤70% relative abundance). Given the oligotrophic nature of glacier surfaces, the predominance of a single highly competitive taxon is not unexpected (Winter et al., 2010). However, the high viral control of bacterial production would imply that a numerically-dominant successful competitor would likely suffer the winner's fate in falling victim to its associated viruses. The continued dominance of a singular *Janthinobacterium* OTU throughout is therefore remarkable and initially challenges the "kill the winner" paradigm (Winter et al. 2010).

Virus-to-bacterium ratio and bacterial production were negatively correlated, suggesting that resources accessible to bacteria were not transferred to the viruses to maintain viral control of the population, but were instead allocated to the bacteria and most likely to defence against viral attack. *Janthinobacterium* can produce extracellular vesicles (Mashburn-Warren et al. 2008), which have multifarious roles, including as antiviral defence (Biller et al. 2014). Since viruses cannot distinguish between host cells and vesicles produced from the outer membrane of the host cell, vesicle-producing bacteria produce decoys that distort the host-virus contact rate. We argue that extracellular vesicles help defend numerically dominant bacteria against viral attack in the glacial photic zone by decreasing host-virus contact rates. This notion was further supported by electron microscopy, which showed that outer membrane vesicles producing bacteria (Figure 1) were common in all examined microcosms.

Consequently, our results suggest that supraglacial viruses exert strong control on bacterial production, abundance and diversity within the supraglacial environment, by means of prolonged longevity of viral particles. Dominant bacterial taxa appear to evade viral control by directing resources to the production of outer membrane vesicles, thereby decreasing their contact rate with viruses and ensuring their continued dominance. Thus, we propose that a delicate interplay of bacterial and viral strategies affects biogeochemical cycling upon glaciers and, ultimately, downstream ecosystems.

KEY WORDS: GLACIER, MELTWATER, VIRAL ECOLOGY, BACTERIAL DIVERSITY, VESICLES

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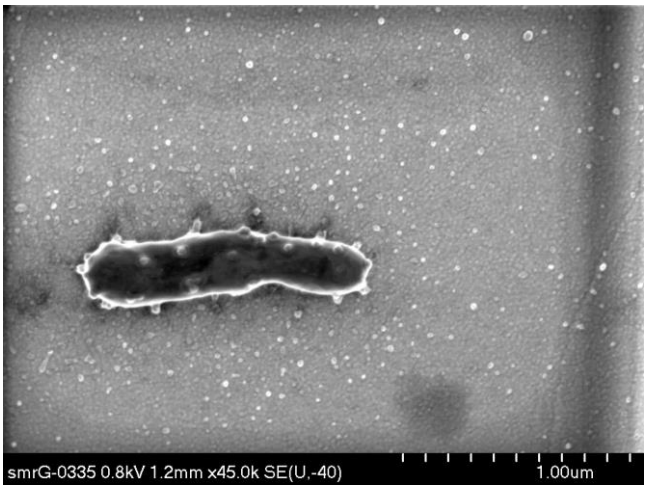

Fig. 1. FE-SEM micrograph of a bacterium producing outer membrane vesicles. Uncoated sample stained with 5% uranyl acetate for 3 min.

Lecture B-07

METAGENOMICS OF PERMAFROST - KEY FOR PALEOECOLOGY

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The late Pleistocene Icy complex and lake-alluvial sediments are widespread within the Kolyma-Indigirka lowland, North-East Siberia (152-162°E, 68-72°N). We have previously found that on this territory, the epigenetically frozen sediments of lake, lake-alluvial and marine origin contain biogenic methane, whereas in the sincryogenic late Pleistovene Icy complex, methane was absent. To investigate the reasons of such particularA specific distribution of methane and methane forming microorganisms in geological cross-section raises questions, why in the sediments of the Late Pleistocene Ice complex on this territory methane is not presented as well as methanogenic activity. To answer this, question we used a the new approach - the metagenomic analysis.

We report the about results of the comparative metagenomic analysis of the two permafrost samples of different origin but aand the very similar in ages – 32000 years. Sample -IC4 corresponds to the permafrost sediment of thea lake originating in from the Panteleikha River floodplain: DH-2007/4; depth, _22.5 m; ~ 2 mmol CH4/kg; 30,696 years. Sample-IC8 corresponds to the sediment was sampled from the late Pleistocene Ice Complex aton the Omolon River: DH-2007/2; depth, 6 m; with no methane detected. The age of the second sample was estimated to beassessed as 32,000 years, due to the origin of the earlier described outcrop.

Statistical analysis of the metagenomes was performed to compare the community composition and functional profiles of the permafrost samples. The differences between proportions were analyzed using the Newcombe-Wilson method with Benjamini-Hochberg FDR correction and 95% confidence interval.

The class-level comparison of the microbial community structure of the samples reveals *Proteobacteria* and *Actinobacteria* are overrepresented taxa. More detailed analysis shows that *Mycobacterium* (aerob *actinobacteria*), *Bradyrhizobium* (facultatively anaerobic, *alphaproteobacteria*), *Rhodopseudomonas (alphaproteobacteria),* and *Hyphomicrobium* (aerobe, *actinobacteria*) prevail in the first metagenome, but *Conexibacter (actinobacteria*), *Streptomyces (actinobacteria*), *Nakardiodes (actinobacteria*), and *Frankia (actinobacteria*) are more spread in the second metagenome. Thus, the domination of the genera might be considered as distinctive features of the metagenomes IC4 and IC8, respectively. Methanogenic archaea contribute up to 0.5 % to the microbial communities. *Methanosarcinaceae* and *Methanobacteriaceae* are the most abundant families detected in both metagenomes. Meanwhile, methanogenic arachaea are overrepresented in sample IC4 but not inrather IC8. This suggestsmeans that deposits were formed under different paleoecological conditions .

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KEYWORDS: PERMAFROST, METAGENOMES, METHANE, ARCHAEA

Lecture B-08

SULFATE-REDUCING BACTERIA IN ARCTIC GRYOPEGS

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The dissimilatory sulfate reduction is the most important anaerobic mineralization pathway in cold marine habitats. A study of the diversity and distribution of sulfate-reducing bacteria (SRB) in cold ecosystems using molecular techniques revealed a wide variety of sulfate-reducers belonging to the genera *Desulfovibrio, Desulfosarcina, Desulfotomaculum, Desulfobulbus, Desulfobacter, Desulfobacterium, Desulfofaba* and *Desulfotalea/Desulforhopalus*, while *Desulfovibrio* and *Desulfotalea / Desulforhopalus* were dominant (Sahm et al., 1999; Purdy et al., 2003; Karr et al., 2005). Microbiological studies in permafrost and low-temperature marine sediments showed the presence of the SRB population, and their number reached 10^6 cells/cm^3 (Knoblauch et al., 1999; Rivkina et al., 2000). Cryopegs are defined as layers of unfrozen ground that are perennially cryotic (forming part of the permafrost) in which freezing is prevented by freezing point depression due to the dissolved-solids content of the pore water (Gilichinsky et al., 2005). Arctic cryopegs are characterized by subzero ambient temperatures and high content of sulfates (0.62-3.84 gl^{-1}); however,the initial attempts to obtain the pure culture of sulfate-reducing bacteria were unsuccessful. *Desulfovibrio arcticus* strain $B15^T$ was the first reported sulfate-reducing bacterium from a cryopeg within permafrost (Pecheritsyna et al., 2012). Here, we study he abundance and distribution SRB inhabiting cryopegs of Yamal Peninsula -extreme ecosystems characterized by high salinity in combination with subzero temperature.

The Yamal Peninsula (Northern Russia) is located in the region of continuous permafrost. Cryopegs were found in the mouth of the river Yaroyaha at depths ranging from 5 to 120 m. Samples were characterized by neutral pH, water samples from boreholes 1Y, 2Y and 3Y characterized by salinity ranged from 14 to 77.2 g l^{-1}.We used both a standard microbiological method of serial dilutions and the SYBR green I real-time PCR (qPCR) assay to quantify total numbers of functional dissimilatory (bi)sulfite reductase (DSR) gene for quantification of SRB in cryopeg samples. Standard primers set DSR1F/5R/DSR4R targeting *dsrAB* gene was used to determine the environmental abundance of SRB in the cryopegs samples.

SRB were detected in the order of2 .7×10^2 to 6.9×10^3 *dsr* gene copy number per ml. This result is in accordance with data estimated by the serial dilutions method (2×10^2to3×10^3cells/ml). Also, qPCR assay was used to estimate the efficiency of different enrichment conditions of the Yamal cryopegs samples. Psychroactive halophilic SRB strain $K3S^T$ was isolated from 3Y sampleand characterized.The cells of $K3S^T$ strain were Gram-negative vibrions, had size of 2 × 0.4-0.5 µm, and were motile due to one polar positioned flagellum. They were positive for desulfoviridin as a bisulfite reductase. Strain $K3S^T$ grew at temperatures of -2–36°C (26˚C optimum) and at salinity of 0.5–4.0% (2% optimum). The strain was able to use hydrogen plus acetate, formate, ethanol, lactate, alanine, pyruvate, and fumarate as electron donors. Utilized electron acceptors included sulfate, sulfite, thiosulfate, elemental sulfur, DMSO and Fe^{3+}. Analysis of the 16S rRNA gene sequence revealed that the isolated organism belonged to the genus *Desulfovibrio* with the closest relatives *Desulfovibrio ferrireducens* $61T^T$ (97.4 % similarity), isolated from Arctic fjord sediments.

The genotypic and phenotypic data strongly support recognition of the strain as a representative of a novel species within the genus *Desulfovibrio,* for which the name *'Desulfovibrio algoritolerans'* with type strain $K3S^T$was proposed. Also it was shown that the new bacterium as previously described *D. arcticus* $B15^T$ was able to grow and generate sulfide at *in situ* temperature of -2°C.

These results suggest that in the microbial communities of Arctic cryopegs sulfate reducers perform terminal stage of organic matter decomposition similarly to marine low-temperature ecosystems.

KEYWORDS: SULFATE-REDUCING BACTERIA, ARCTIC CRYOPEGS, *DESULFOVIBRIO* SP.

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Lecture B-09

DIVERSITY OF *cbbL*, *nifH* AND *pufLM* GENES IN SOILS AROUND THE PRINCESS ELISABETH STATION, SØR RONDANE MOUNTAINS, ANTARCTICA

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INTRODUCTION

In Antarctica, the presence of native flowering plants is restricted to the Peninsula. In continental Antarctica, photosynthesis by Cyanobacteria is generally thought to be the main primary source of organic carbon. Many cyanobacterial species are also capable of fixing nitrogen, allowing them to survive and prosper in almost every environment, including the adverse conditions of Antarctica. Several studies, including our own findings, however, have shown that Cyanobacteria are sometimes only scarcely present in the terrestrial Antarctic microbial communities. Furthermore, both carbon and nitrogen fixation require a considerable amount of ATP. In Antarctica, sunlight – an abundant energy source during the Antarctic summer – may represent an important resource to generate this ATP. Some bacteria, for example, are known to use rhodopsin-like pigments to exploit sunlight, whereas aerobic anoxygenic photosynthetic bacteria can use bacteriochlorophyll to harvest light energy that is then stored as ATP. We explored the hypothesis that, in the ice-free regions of continental Antarctica, other primary producers and bacteria that exploit solar energy may contribute to carbon and nitrogen fixation, in addition to Cyanobacteria.

METHODS & MATERIALS

The presence and diversity of non-cyanobacterial prokaryotes that possess these properties was studied in four terrestrial samples gathered in the vicinity of the Belgian Princess Elisabeth Station (Sør Rondane Mountains, Queen Maud Land, East Antarctica). A culture independent approach by Illumina MiSeq amplicon sequencing of *cbbL* (carbon fixation, RuBisCO type I), *nifH* (nitrogen fixation), and *pufLM* and proteorhodopsin genes (light-harvesting) was used. After curation, sequences were placed in phylogenies with existing sequence data to reveal phylogenetic affiliations.

RESULTS

Proteorhodopsin genes failed to amplify from all tested samples. Illumina sequencing extended the functional genes' sequence datasets, previously obtained by performing PCR clone libraries, by several orders of magnitude. The clone library rarefaction analysis showed that no saturation was reached for any gene, indicating that a large proportion of the diversity remains unknown using this approach. Illumina results, also revealing the diversity obtained with clone library analysis, additionally uncovers part of the previously unknown diversity, thus improving our knowledge in the diversity of the functional genes.

DISCUSSION

Overall, the data obtained with Illumina suggest that, in soils in the proximity of the Belgian Princess Elisabeth Station in the Sør Rondane Mountains, a broad and unknown diversity of bacteria harboring *cbbL*, *nifH* and *pufLM* genes are present. Non-cyanobacterial *cbbL* genes were dominant to cyanobacterial ones, indicating that their hosts may contribute significantly to the input of organic matter in the oligotrophic Antarctic biosphere. Diversity of *nifH* was low, as mostly Cyanobacteria affiliated sequences were recovered from the sequencing data, suggesting that, although they may only be present in small numbers, diazotrophic Cyanobacteria supply most of the fixed nitrogen in Antarctic soils. Furthermore, aerobic anoxygenic phototrophic bacteria were shown to be present in Antarctic soils. Photoheterotrophy, using the abundant sunlight of the Antarctic summer, may thus indeed be a useful life strategy in this challenging environment.

KEYWORDS: ANTARCTICA, ILLUMINA, CARBON FIXATION, NITROGEN FIXATION

Lecture B-10

HOST-VIRUS INTERACTIONS IN A FRIGID, HYPERSALINE ANTARCTIC LAKE REVEALED BY METAPROTEOMICS

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Deep Lake is a marine derived, hypersaline system in Antarctica that remains perennially ice-free with water temperatures dropping to -20°C. These harsh environmental conditions have led to a low complexity microbial community, completely dominated by members of the haloarchaea, including four isolated species (tADL, DL31, *Hrr. lacusprofundi* and DL1) that account for ~72% of the lakes cellular population. Genomic sequencing and analysis of the four isolated species combined with metagenomics have revealed an unprecedented level of inter-genera exchange of long (up to 35 kb) stretches of identical DNA. However, despite the rampant, promiscuous exchange of DNA, distinct haloarchaeal lineages appear to prevail in the lake by virtue of their unique capacities for niche adaptation (Williams et al. 2014, DeMaere 2013). With no apparent metazoan grazers present in the lake, viruses are hypothesised to play a dominant role in shaping the microbial community of Deep Lake. In this present study we applied metaproteomics for the first time on a hypersaline environment and combined it with in-depth genomic and metagenomic analysis of Deep Lake CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) and BREX (Bacteriophage Exclusion; Goldfarb 2015) systems to elucidate host-virus interactions.

Shotgun metaproteomics was performed on Deep Lake biomass from 5 distinct depths, captured by sequential filtration through 3 µm, 0.8 µm and 0.1 µm filters during the Antarctic summer of 2008/2009. All identified proteins were manually annotated and grouped into taxonomic and functional categories. We characterized CRISPR systems of the four genomes and the Deep Lake metagenome and used CRISPR spacer and repeat sequences to identify sources of invading DNA.

The Deep Lake metaproteome comprised around 1100 detected proteins. A striking feature was the identification of multiple, highly abundant cell surface proteins with a high degree of sequence variation compared to the genomes of the isolate species (“variants”). E.g. we identified 6 distinct proteins all matching the main S-layer component of tADL. Furthermore we detected variants for archaella (archaeal flagella), pili and other cell surface proteins. Multiple viral proteins were detected with sequence similarity to other, mainly haloarchaeal viruses. Functional CRISPR loci could be identified in the genomes of all four isolated species and CRISPR-associated (Cas) proteins were detected for two of them. CRISPR spacers could be linked to different sources of invading DNA, with most, but not all spacers targeting viruses. We detected one BREX protein (PglX) for *Hrr. lacusprofundi*. Some detected proteins, including cell surface proteins, were encoded on metagenome contigs together with putative viral genes.

The detection of multiple protein variants for cell surface structures like S-layer and archaella is indicative of phylotypes that are present in the lake. Introducing variation in cell surface structures likely provides the haloarchaeal populations with a way of evading viral infection. Consistent with this is the presence of a diverse viral population in Deep Lake. We detected proteins from at least eight distinct haloarchaeal viruses (eight major capsid proteins), with some proteins confirming active viral life cycles (e.g. prohead protease). Furthermore, the CRISPR spacer analysis revealed that some viruses infect multiple species (broad host range). In addition to the acquired cell surface variation, haloarchaeal host cells have employed active CRISPR and BREX systems as defense against viral infection.

The presence of cell surface genes on metagenomic contigs together with putative viral genes, and the high degree of sequence variation observed in many cell surface proteins, suggests that viruses are involved in the acquisition, mutation and distribution of cell surface variants within the haloarchaeal populations. Overall, we were able to identify and describe a complex network of virus-host interactions, revealing a pivotal role of viruses in shaping the microbial community in Deep Lake (Tschitschko et al., submitted).

KEYWORDS: METAPROTEOMICS, VIRUS-HOST NETWORKS, CRISPR, HYPERSALINE SYSTEMS, HALOARCHAEA

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Lecture B-11

MICROBIAL COMMUNITIES OF ANTARCTIC SOIL AND LITHIC HABITATS

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Climatic changes are predicted to disproportionately affect the delicate environments found at high latitudes and altitudes across the globe. The disturbance of polar desert ecosystems may alter key biogeological processes that may be solely mediated by micro-organisms in these threatened biomes. The Antarctic Dry Valleys are a series of hyperarid polar deserts, are highly oligotrophic, experience near-constant below-freezing temperatures, and are critically low in bioavailable moisture. Increases in air and surface soil temperatures, coupled with augmented ultra-violet irradiation receipt, are predicted to supply local microbial communities with previously unattainable levels of moisture and nutrients as regional glacial and permafrost ice-melt intensifies (Fountain et al. 2014). Predicting the responses of distinct bacterial communities to perturbations in available moisture content is the critical focus of this study. Here replicated microbial fingerprinting, terminal restriction fragment length polymorphism (T-RFLP), and 454 tagged-pyrosequencing, in combination with multivariate statistical analyses, were applied to address this knowledge deficit. Soil and lithic samples were retrieved from Victoria Valley and comprised four edaphic habitats; hypoliths (*n*=14), endoliths (*n*=10), mats (*n*=4) and surface soils (*n*=5). Samples were collected from hyperarid (soils and endoliths) or inundated (hypoliths and mats) sites so that the role of unique moisture regimes on bacterial diversity and community structure could be assessed. This study presents evidence supporting the concept of ecological niche partitioning between local desert habitats. Multivariate analyses of bacterial 16S rRNA gene-defined communities (hypervariable regions V3 – V5) showed that edaphic niches were significantly distinct in structure. However moisture content did not appear to be a significant delineator of general bacterial communities on the basis of the results presented here. Cyanobacterial populations were not delineated by either habitat or moisture content on the basis of the T-RFLP fingerprinting data. Pyrosequencing data of representative samples (*n*=4) were analysed using MOTHUR and revealed that soil communities were highly diverse relative to specialised habitats at the bacterial phylum-level. Soil communities are predicted to 'seed' the development of specialised communities, which supports the concept of species recruitment in desert systems (Makhalanyane et al. 2013). The sequence data presented here suggests that more than 13 bacterial phyla were represented across all habitats, suggesting that accounts of microbial diversity in the Dry Valleys may have been underestimated previously (Pointing et al. 2009). Moisture content alone was less significant in determining local bacterial diversity patterns according to the fingerprinting and pyrosequencing techniques applied here. The sequence data revealed a strong positive correlation between cyanobacterial abundance and moisture content. Oscillatorian cyanobacterial genera *Leptolyngbya* and *Phormidium* were highly abundant and dominated all samples. The number of cyanobacterial signals assigned to the hypolithic community were notably higher than were found in communities obtained from xeric sites. These data suggest that higher levels of local moisture content may influence cyanobacterial population structures and lead to increased proliferation of the phylum in this hyperarid desert, as has been predicted previously (Wood et al. 2008). Taken together, these results appear to suggest that deterministic processes supersede stochastic events in determining diversity patterns across this polar desert. This may be critical in terms of global climate change as rapidly shifting environmental parameters may lead to detrimental alterations to local desert community structures.

KEYWORDS: PYROSEQUENCING, DETERMINISTIC, STOCHASTIC, ANTARCTICA, MOISTURE, HABITAT

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Poster B-01

BIODIVERSITY AND DISTRIBUTION OF POLAR FRESHWATER VIRUSES

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We report a metagenomic analysis of Arctic freshwater viral communities and we have conducted a comparative analysis of previously published freshwater viromes. The previously unexplored Arctic viromes were similar in their taxonomic composition, mainly dominated by unknown and ssDNA viruses. However, most polar contigs obtained were previously unknown, and we were able to find contigs representative of the recently described RNA-DNA hybrid viruses. Arctic viral communities were found to share genes, and we detected three shared genomes. Global viromes from the same environment were most similar to each other, but showed some degree of coarse grain genetic overlap to other environments. The Arctic Ocean virome clearly separated from the Arctic samples, but also from the other freshwater viromes, and results were not supportive of a latitudinal diversity gradient existing in freshwater viromes. While similar in their taxonomic distribution, Arctic and Antarctic viromes differed at the fine grain genetic level, indicating that they are dominated by different viral lineages. Yet unexpectedly, we were able to find circular contigs in both environments showing sequence similarities greater than 90%.

KEYWORDS: VIRUS, ARCTIC, METAGENOMICS, LAKE

Poster B-02

ECOLOGICAL CONNECTIVITY SHAPES VIRAL ASSEMBLAGES AND VARIABILITY IN ANTARCTIC ENVIRONMENTS

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Viruses play a pivotal role in the control of microbial communities by the selective pressure they exert on their hosts or the horizontal transfer of genes between different microorganisms. We will present a metagenomic survey of viruses in terrestrial ecosystems surrounding Limnopolar lake (Byers Peninsula, Livingston Island) including soil, cyanobacterial mats and the sediment of the lake. The distribution of dominating ssDNA viral genomes provides insights to understand how these environments are interconnected. In order to achieve a more general view of viruses in Antarctica we performed a metagenomic analysis of RNA viruses in Limnopolar lake along three years. These RNA viromes showed lower diversity than the corresponding DNA viromes, with a dominance of a few viral species belonging to the order picornavirales and likely infecting protists. Deep sequencing of the viral genomes allowed us to analyze for the first time viral RNA quasispecies structures in natural aquatic ecosystems. This study establishes the impact of ecological connectivity between terrestrial and aquatic ecosystems on the genetic diversity within viral species.

KEYWORDS: VIRUS, ANTARCTICA, RNA QUASISPECIES, CYANOBACTERIAL MAT, LAKE

Poster B-03

ATTEMPTED ISOLATION OF *ACIDOBACTERIA* FROM ANTARCTIC PERMAFROST

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Permafrost samples from Taylor Valley, Antarctica are dominated by bacteria from *Acidobacteria* Gp6 as determined by 16S rRNA gene clone libraries (Bakermans et al. 2014). While *Acidobacteria* are likely important members of permafrost communities, little is known about *Acidobacteria* because they are notoriously extreme oligotrophs and difficult to cultivate. We are attempting to isolate *Acidobacteria* from Taylor Valley permafrost using very dilute media (Davis et al. 2005; George et al. 2011; Joseph et al. 2003).

Permafrost samples were diluted in ice cold sterile water and spread on plates of dilute media prior to incubation at 10 and 0°C. Media contained 1/200th strength nutrient broth (dN), trypticase soy broth (dTS), or R2 broth (dR2) with either gellan gum or noble agar added to solidify the medium; Luria agar was included as a control. The number of colonies increased over time with the most growth evident on Luria agar (32,000 cfu/g at 10°C and 20,000 cfu/g 0°C after 70 days). Growth on diluted media ranged from 10 to 7748 cfu/g with the most colonies evident on dTS and the fewest on dR2. Number of colonies detected on dilute media continues to increase over time with 85,000 cfu/g detected on dTS after 412 days of incubation at 10°C. In general, more colonies were evident on media solidified using gellan gum, however gellan gum dehydrated quickly at these temperatures which is problematic for long-term incubations.

Since *Acidobacteria* typically grow slowly forming small colonies, plates were also examined at 7× magnification with a dissecting scope. Microcolonies ranging in diameter from 0.1 to 0.6 mm were evident on dTS after 52 days of incubation at 10°C. Numbers of microcolonies continue to increase with time and were seen on dN after 270 days of incubation at 10°C. Microcolonies from dTS and dN were re-streaked for isolation and testing by PCR and sequencing of the 16S rRNA gene to verify the identity of isolates. To date, 13 isolates have been successfully subcultured on dilute media supplemented with vitamins and minerals, but none are *Acidobacteria*. Two isolates have been identified as actinobacteria from the genus *Janibacter.*

Molecular techniques are also being used to track the growth of *Acidobacteria* on dilute media. After 110 days of incubation at 10°C and 300 days of incubation at 0°C, all cells were collected from plates inoculated with the lowest dilution (10^{-1}) of soil samples and total DNA was extracted for assessing the presence of *Acidobacteria* by PCR (Navarrete et al. 2013). *Acidobacteria* were detected in DNA from cells collected from dN and dTS plates, but not from Luria agar or dR2, at both incubation temperatures.

In summary, *Acidobacteria* from Antarctic permafrost are capable of growing on dilute media at low temperatures in the laboratory. Isolates obtained this way are likely cold-adapted oligotrophs that are successful in this low-nutrient environment. Ongoing work will determine the prevalence of *Acidobacteria*, attempt the subculture of *Acidobacteria*, and identify microcolony isolates. These data will facilitate the cultivation of *Acidobacteria*, and other oligotrophic bacteria, from permafrost.

KEYWORDS: COLD ADAPTATION, PSYCHROPHILIC, ANTARCTIC, PERMAFROST

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Poster B-04

VIRUS GENOMES FROM GLACIAL ENVIRONMENTS REVEAL NOVEL VIRUS GROUPS WITH UNUSUAL HOST INTERACTIONS

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Microbial communities in glacial ecosystems are subjected to strong viral pressures and some of the highest infection rates in the literature, making viruses a likely driver of bacterial evolution. Here we analyse putative virus genomes assembled from cryoconite hole metagenomes from Svalbard and the Greenland Ice Sheet to assess the potential hosts and functional role viruses play in these habitats. Analysis of virus marker genes revealed a wide range of viruses are present which infect bacteria, cyanobacteria, eukaryotic algae and amoebae. Whole genome comparisons revealed the majority of genomes formed 12 novel virus groups, two of which contained multiple lysogenic bacteriophage members with plasmid-like properties, including a group of satellite viruses. Interestingly, one of the lysogenic phage also encoded a complete CRISPR/Cas adaptive bacterial immune system with spacers to target another phage, indicating for the first time that a phage may protect its bacterial host from other invading phage using this system.
Together these results suggest that highly novel and diverse groups of viruses are present in glacial environments, some of which utilise very unusual life strategies and genes to control their replication and maintain a close association with their hosts.

KEYWORDS: VIRUS, BACTERIOPHAGE, CRYOCONITE, METAGENOMICS

Poster B-05

PROKARYOTIC COMMUNITIES ACROSS A GEOCHEMICAL AND TEMPERATURE GRADIENT ON AN ANTARCTIC ACTIVE VOLCANO (DECEPTION ISLAND)

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Polar volcanoes offer unique conditions of temperature and geochemical gradients that promote the growth of metabolically diverse microorganisms. Volcanoes in Antarctica are mainly distributed in three continental sites and in circumpolar islands, such Deception Island in the South Shetland Archipelago. This island is an active polar stratovolcano located on a rift along the Bransfield Strait. Glaciers cover more than a half of Deception and a series of geothermal sites are distributed along the island, which results in a surface temperature that ranges approximately between 100°C to -10°C in the summer. Sulfur and iron compounds, CO_2 and water vapor are commonly emitted by fumaroles in Deception, and as well as the temperature, provide a gradient that creates a mosaic of environmental conditions and opportunities for microbial colonization. The aim of this study was to describe the microbial communities along a temperature and geochemical gradient on two geothermal sites at Deception Island, Antarctica, through 16S rRNA gene sequencing approach. The sediment samples were collected on a superficial three points transect along the geochemical and temperature gradient, in triplicates, next to the fumaroles on Fumarole Bay and Whalers Bay geothermal fields, totalizing 18 samples. Total microbial DNA was extracted from 10g of sediment using PowerMax Soil DNA kit (MoBio, USA) and amplified with bacterial primers 341F and 785R that corresponds to the V3-V4 regions of 16S rRNA gene. The library was constructed through an index PCR and the amplicons were sequenced using Illumina Miseq technology. The sequencing resulted in 1,253,576 reads with approximately 460 bp. These reads were distributed along 15 sediment samples, due to non-amplification of triplicate samples from the highest environmental temperature (98°C) of Fumarole Bay geothermal site. For this reason, these samples were sequenced with the archaeal primers 519F and 1017R and exhibited Crenarchaeota (83,76%) and Euryarchaeota (8,18%) as dominant Phyla and *Aeropyrum*, *Vulcanisaeta*, *Haloferax*, *Pyrococcus*, *Ignicoccus* and *Desulfurococcus* as abundant genera, most of them represented by hyperthermophilic archaea, with optimal growth around 90°C. The dominant bacterial Phyla of the other 15 samples were Proteobacteria (43,75%) and Bacteroidetes (17,11%). The remaining sequences were classified into Verrucomicrobia (8,07%), Actinobacteria (7,86%), Planctomycetes (4,97%), Candidate division OD1 (2,09%), Acidobacteria (2,00%), Chloroflexi (1,91%), Candidate division TM7 (1,54%), Gemmatimonadetes (1,19%), Cyanobacteria (0,69%), Armatimonadetes (0,43%), Firmicutes (0,38%), Elusiomicrobia (0,21%), and other 32 different Phyla (total of 1,40%) or Unclassified (5,74%). 43,37% of these sequences were classified into 858 different genera, in which the fifteen most abundant genera (42,25%) included *Thalassomonas*, *Flavobacterium*, *Luteolibacter*, *Ferruginibacter*, *Rhodanobacter*, *Dokdonella*, *Polaromonas*, *Chthoniobacter*, *Planctomyces*, *Simplicispira, Gemmatimonas, Arenimonas, Caldithrix, Patulibacter* and *Blastopirellula. Thalassomonas* and *Caldithrix* genera were predominantly distributed into the samples with the highest environmental temperatures, which were closely associated with the fumaroles on the Fumarole Bay and Whalers Bay geothermal field. On the other hand, *Flavobacterium* and *Luteolibacter* genera were distributed in samples with the lowest environmental temperatures, which were located close to the glaciers at the both studied sites. The geochemical analysis of the sediment shown a different distribution of the elements according to the distance from the fumaroles: samples near to the fumaroles were rich mainly in Iron, Sodium, Magnesium and Sulfate, while samples near to the glaciers exhibited higher values of total Nitrogen, Ammonium and Nitrate. The results show that rapid shifts in environmental temperatures and geochemical compositions over small spatial scales can act as a strong selective pressure and affect directly the structure of the microbial communities. The co-occurrence of hyperthermophilic archaea commonly found in deep-sea hydrothermal vents with metabolically diverse cold-adapted bacteria, such photosynthetic, chemolithotrophic and heterotrophic, represents a distinguished microbial composition that reflects the singular polar and shallow marine geothermal site that is Deception.

ACKNOWLEDGMENTS: FAPESP, CNPq-PROANTAR.

KEYWORDS: POLAR VOLCANOES, GEOTHERMAL FIELD, ANTARCTICA, DECEPTION ISLAND, TEMPERATURE AND GEOCHEMICAL GRADIENTS, MICROBIAL COMMUNITIES, 16S rRNA SEQUENCING, ADAPTATION, DIVERSITY, PHYLOGENETICS

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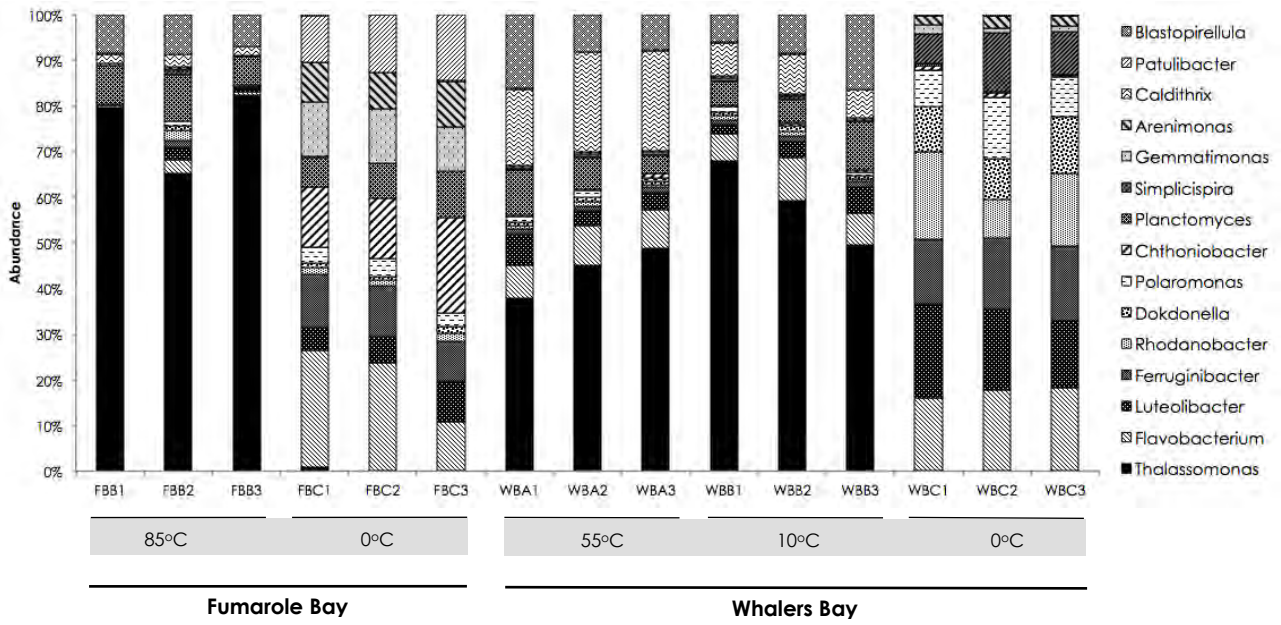

Fig. 1. Distribution of the fifteen most abundant bacterial genera across temperature gradient on geothermal fields of Fumarole Bay and Whalers Bay, Deception Island, Antarctica.

Poster B-06

BIOLOGICAL SOIL CRUST ALGAE IN THE POLAR REGIONS – BIODIVERSITY, GENETIC DIVERSITY AND ECOSYSTEM RESILIENCE UNDER GLOBAL CHANGE SCENARIOS

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Biological soil crusts represent an association of soil particles and various organisms. They are formed by cyanobacteria, green algae, lichens, bacteria, microfungi and bryophytes. These communities act as ecosystem developers in nutrient-poor habitats and occur mostly in extreme habitats such as hot and cold semiarid/arid areas. Biological soil crusts have important ecological functions in primary production, nitrogen fixation, soil stabilization, water retention and biogeochemical cycles. Since biological soil crusts are badly studied in the polar regions, the main goal of this interdisciplinary project is a precise evaluation of the biodiversity of green algae and cyanobacteria in samples isolated from the Arctic (Spitzbergen) and the Antarctic Peninsula.
The first expedition took place in Spitzbergen in August 2014. The research areas were situated around the two localities Ny-Ålesund and Longyearbyen. The second field study was in January 2015 on Livingston Island of the Antarctic Peninsula. During both expeditions single point measurements for vegetation surveys (areal coverage of crusts) were undertaken and samples for the determination of the biodiversity and ecophysiology of green algae and cyanobacteria taken. The organisms will be identified by both morphological and genetic analyses. The effects of temperature and water stress on growth and photosynthesis tolerance widths of several taxa will be experimentally studied..The adaptation to changing environmental conditions will also be molecularbiologically monitored using RNAseq – under the experimental conditions and in the field (meta-transciptomics).
On Spitzbergen a surprisingly high areal coverage by biological soil crusts was determined. For example, on average 50% of the soil was crust covered in Ny-Ålesund. The studied areas differed strongly from each other by composition of the lower vegetation and areal coverage (18% - 89%). The data presented and planned experiments will indicate whether and how global change in polar regions influence the ecological performance of biological soil crusts. Thus, it will be possible to make predictions on the future significance of ecological functions of these pioneer communities.

KEYWORDS: ARCTIC, ANTARCTICA, GREEN ALGAE, TERRESTRIAL, VEGETATION SURVEY

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Poster B-07

DIFFERENTIAL ABUNDANCE AND EXPRESSION OF ANTARCTIC SOIL MICROBIAL COMMUNITIES: A METATRANSCRIPTOMIC ANALYSIS OF TAXONOMIC AND FUNCTIONAL DIVERSITY

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INTRODUCTION

The McMurdo Dry Valleys (MDV) are an extreme polar desert region of Antarctica, consisting of exposed soils, relatively free of snow and ice cover. This unique region is an exclusively microbial ecosystem, lacking higher plant and animal residents. As such, MDV soils are an exceptional study site to describe microbial processes free from many confounding factors common to other terrestrial ecosystems. Microbial life of MDV soils is known to be active during the brief Antarctic summer (Zeglin et al. 2009, Schwartz et al. 2014), taking advantage of ephemeral glacial melt water inputs and warmer temperatures. Climate change is increasing warming events in this region, and thus increasing soil moisture and mobilized nutrients in the soils (Fountain et al. 2014). However, little is known about what functions the resident soil microbes perform, and how these functions change with increasing water and nutrient availability. The experimental research presented here therefore furthers understanding of extreme terrestrial ecosystem dynamics and climate change effects on microbial systems.

METHODS

This project uses a metatranscriptomic approach to investigate summer soil microbial activity in the MDV. Water and organic matter *in situ* treatments were included to increase soil moisture by 10%, providing an experimental basis to consider community responses to environmental pulses relative to unamended controls. The total mRNA transcripts of soil microbial communities were sequenced using Illumina HiSeq and resulting sequences were annotated by MG-RAST, using the M5NR and SEED Subsystems databases for taxonomic and functional annotation, respectively. Differential abundance of taxonomy and differential expression of functional reads were analyzed using the phyloseq and DESeq2 packages in R.

RESULTS

Differential abundance. Responses to treatments were variable within individual phyla; some members of a phylum may decrease abundance significantly while other members of the same phylum increase significantly. Key soil bacterial phyla *Actinobacteria* and *Proteobacteria* had the greatest responses to water treatments relative to controls, each with significantly positive and negative abundance changes. For organic matter treatments, members of *Firmicutes* had the greatest differential abundance increase, consistent with other studies of nutrient additions to these soils (Schwartz et al. 2014, Van Horn et al. 2014). As in water treatments, *Actinobacteria* and *Proteobacteria* also had some of the strongest abundance responses to organic matter addition relative to controls.

Differential expression. Contrary to the variable responses seen in differential abundance measures, transcript expression tended to show clearer trends at high level functional categories: transcripts within a functional category were either over- or under-expressed relative to controls. Under-expression was the only significant response in water addition samples relative to controls, with Protein Metabolism transcripts exhibiting the largest reduction. For organic matter additions, many functional categories were also under-expressed relative to controls. Closer investigation indicates these transcript declines in both treatments largely stems from eukaryotic transcript loss. There were also positive responses in differential expression of organic matter samples, and these positive responses were exclusively bacterial transcripts, from the key soil phyla *Firmicutes*, *Proteobacteria*, and *Actinobacteria*.

DISCUSSION

The treatments of this study were meant to mimic the expected effects of climate change in the MDV, and both water and organic matter additions appear to be stressors to these soil communities. Overall biodiversity and transcript diversity declined in both treatments relative to controls, with members of a few bacterial phyla outcompeting all other taxa.

KEYWORDS: METATRANSCRIPTOMICS, DIFFERENTIAL EXPRESSION, SOILS, ANTARCTICA

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Poster B-08

CHARACTERISATION OF MICROBIAL COMMUNITIES IN WATER TRACKS IN AN ANTARCTIC DRY VALLEY

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Pearse Valley is a cold, arid Antarctic dry valley with mean monthly temperatures ranging –27.4°C to –2.8°C and less than 100 mm of precipitation per year. Water tracks, which are streaks of wetter soils, have been found on the slopes of Pearse Valley, and provide an opportunity to study the effects of water limitation on microorganisms in a polar desert. Due to their low temperatures and low water activity, polar deserts have been used as potential analogues for Martian habitats. Various features on Mars, such as the recurring seasonal low-albedo "recurring slope lineae" that occur on equator-facing slopes in warm seasons, are hypothesised to be related to flowing water (Ohja et al. 2014), and indirect evidence that perchlorate salts may be stabilising transient liquid water at Gale crater on equatorial Mars has been found (Martín-Torres et al. 2015). The water tracks of Pearse Valley have potential as analogues to these potential Martian habitats. To understand the effect of water availability on the microbial community in a cold and arid environment, the microbial communities and activity of the water tracks and dry soils of Pearse Valley are being explored. Of interest are the viable and total biomass, community composition, low-temperature heterotrophic activity, and growth under cold and osmotic stress. 20 isolates have been found and several have been selected for further characterisation. The characteristics and microbial communities of the water tracks will be compared against the characteristics and communities of surrounding dry soil. Insight into the ability of microorganisms to survive in water tracks in a polar desert will help us understand the effects of water availability on life on Earth and potentially wet soils Mars.

KEYWORDS: WATER TRACKS, MCMURDO DRY VALLEYS, ANTARCTICA, POLAR DESERT

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Poster B-09

FUNGAL DIVERSITY IN PERMANENTLY ICE COVERED LAKE FRYXELL, ANTARCTICA

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Introduction: Lake Fryxell in the McMurdo Dry Valleys of Antarctica is a permanently ice covered stratified lake. This endorheic basin is primarily fed by the Commonwealth and Canada Glaciers (77°37'S 163°11'E). Lake Fryxell has a strong sulfide gradient reaching to approximately 1-2 m above the sediment (Lawrence and Hendy 1985). Diverse bacterial communities are found in various parts of this stratified lake (Roberts et al. 2000; Karr et al. 2003; Karr et al. 2005). However, it has been suggested that fungi in Antarctic lakes may simply be remnant isolates that have been deposited in the lakes through glacial run-off and not distinct communities them selves. To test this we determined the diversity of fungal communities in both lake water and glacial in-put. During austral summers 2003, 2004, 2008, 2012, and 2013 culturable fungi were collected from waters at 2 m intervals to the anoxic zone ~1 m above the sediment (12 m) as well as from inputs from both Canada and Commonwealth glacier runoff.
Samples from the 2008 austral field season were used to determine abundance as well as community diversity. Samples of 1L from 7m, 8m, 9m, 11m, and 12m depths were filtered onto black 0.45 sterile membranes in 250ml aliquots. Each filter was placed onto YPD medium (plus antibiotics) and incubated at 4 °C for up to one year. At weekly intervals the filters were photographed and sub-cultures were taken of fungal colonies as the colonies became large enough. Taxonomic placement was determined by either F-ARISA (Slemmons et al. 2013) or by standard Sanger sequencing of the complete internal transcribes spacer (ITS) region. A total of 21 fungal taxons were recovered from Lake Fryxell samples (figure 1) with approximately equal numbers of ascomycetes and basidiomycetes genera represented. The fungal community composition changed with depth, especially between above and below 11m depth. The most abundant fungal species cultured in the 2008 was *Glaciozyma watsonii*, a species that was only found in the upper part of the water column (figure 2) while *Aureobasidium subglaciale* dominated the fungal community below 11m. Neither of these species were found in stream inputs from Canada or Commonweath Glaciers showing that the Lake Fryxell fungal community is not simply remnants from glacial runoff.

KEYWORDS: FUNGAL DIVERSITY; LAKE FRYXELL, ANTARCTICA

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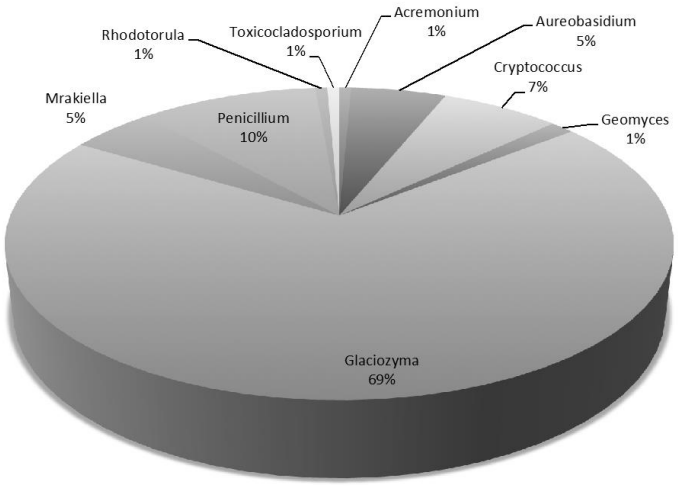

Fig. 1 Diversity of fungal cultures by genus.

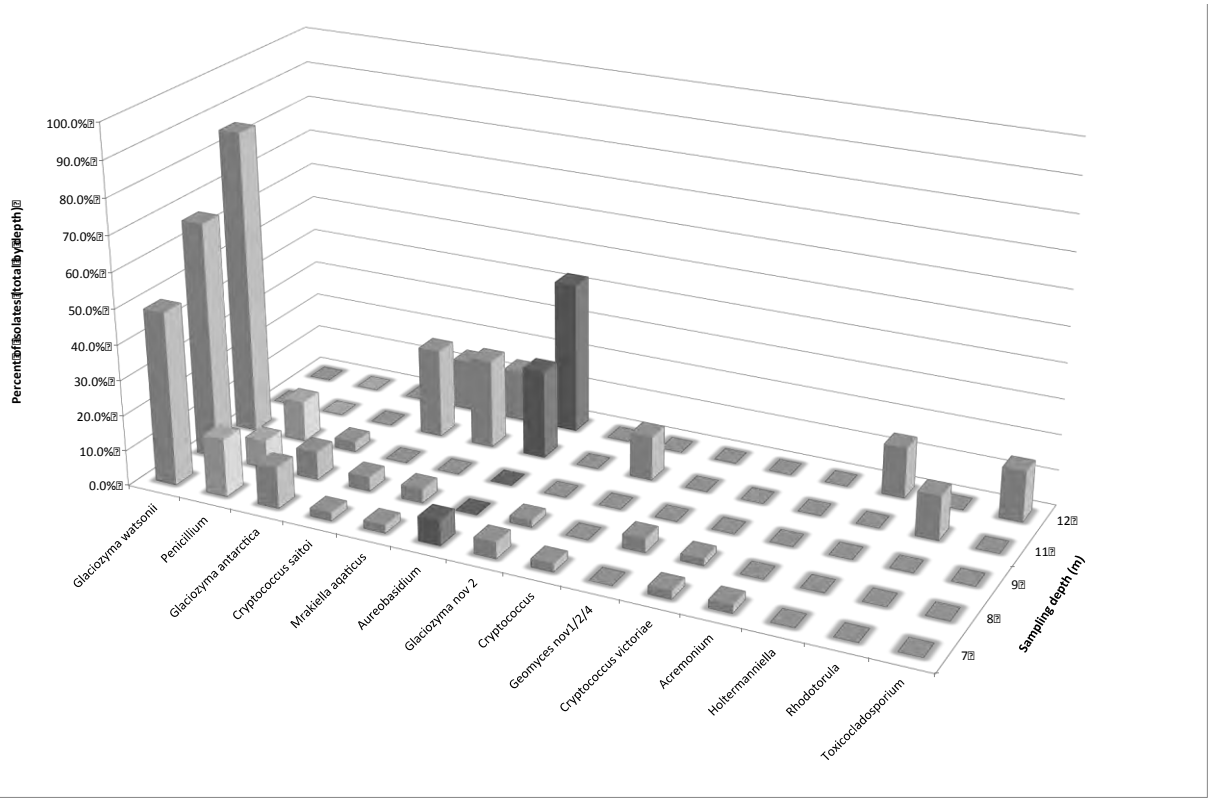

Fig. 2. Fungal community distribution in Lake Fryxell during the 2008 austral season by depth.

Poster B-10

CULTIVABLE HETEROTROPHIC BACTERIA FROM ANTARCTIC PERMAFROST

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INTRODUCTION

Permafrost represents a unique ecosystem for the study the cold-adapted microorganisms. It is characterized by a structural heterogeneity, as it has both horizontal and vertical differences in soil or sediment texture, ice content and organic-matter content. Permafrost is extensively distributed throughout terrestrial Antarctica and in the Arctic, thus representing a major portion of the terrestrial cryosphere. It is a peculiar and unique ecosystem that could allow understanding microbial life strategies in frozen soil as well as the impact of climate change on microbial communities, and their functional roles. However, relatively little is known about microbial life in Antarctic permafrost compared to Arctic and Siberian ones. In this context, the aim of the present study was to establish more suitable methods and culture media for the isolation of heterotrophic bacteria from Antarctic permafrost.

METHODS

Six permafrost samples, collected in two sites in the Northern Victoria Land: namely Boulder Clay (BC-FM, BC-S, BC-I and BC-5) and Edmonson Point (EP1) close to the Italian Antarctic station (MZS), and in the Upper Victoria Valley within the McMurdo Dry Valleys (DY1), had different ice content and lithology. Different isolation procedures were adopted to recover aerobic bacteria: direct plating of suspension, enrichment of cell suspension and natural enrichment. Aliquots were plated on R2A agar, Tryptone Soya Agar (TSA) at different nutrient concentrations (TSA 1%, TSA 50% and TSA 100%) and AcidAgar. Plates were incubated at 4°C and checked for bacterial growth after one month. Bacterial colonies were randomly isolated and purified. A selection of bacterial isolates was identified by the 16S rRNA sequencing.

RESULTS

The highest morphological diversity of bacterial colonies was observed in EP and BC-S samples (by direct plating and enrichment of cell suspension) on R2A and TSA plates. The enrichment of cell suspension was the best method to yield different colony morphology. Conversely, the natural enrichment method was the most appropriate for the recovery of higher bacterial numbers. Exception was the AcidAgar medium on which CFUs ranged from 1.5 x 10^3 to 2.1 x 10^3 *per* gram of permafrost.

A total of 87 strains were preliminarily selected and identified. They were predominantly Gram-positive bacteria within the *Actinobacteria* (28 isolates) and *Firmicutes* (31 isolates), and belonged to the genera *Arthrobacter*, *Micrococcus*, *Microbacterium*, *Cryobacterium*, *Brevibacterium*, *Bacillus*, *Paenibacillus* and *Sporosarcina*. The *Proteobacteria* were less represented (17 isolates) and distributed among the *Gamma*- (13 isolates), *Beta*- (2 isolates) and *Alphaproteobacteria* (2 isolates). The genera were *Psychrobacter*, *Stenotrophomonas*, *Pseudomonas*, *Brevundimonas* and *Polaromonas.*

DISCUSSION

The cultivation on TSA medium was the best method for the recovery of bacteria from Antarctic permafrost samples. Rich media (i.e. TSA and R2A) yielded higher number of bacterial colonies than diluted media (i.e. TSA 50% and TSA 1%) and showed greater morphological diversity. Results from the 16S rRNA sequencing revealed the predominance of *Arthrobacter*, *Brevibacterium* and *Bacillus* members, in line with previous studies. Overall, the composition of the analyzed cultivable fraction was similar to that observed for Siberian permafrost by Steven et al. (2007), reporting the occurrence of the *Firmicutes* (with the three genera *Bacillus*, *Sporosarcina* and *Paenibacillus*), *Actinobacteria* (with the genera *Arthrobacter* and *Micrococcus*) and *Proteobacteria* (genus *Pseudomonas*).

KEYWORDS: PERMAFROST, ISOLATION PROCEDURES, HETEROTROPHIC BACTERIA, BACTERIAL IDENTIFICATION

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Poster B-11

EXPERIMENTAL APPROACH TO THE SCREENING OF PROKARYOTIC ASSEMBLAGE IN ANTARCTIC PERMAFROST

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INTRODUCTION

Permafrost covers more than 25% of the Earth's surface. It is extremely sensitive to global climate warming and its degradation could have dramatic impacts on the entire Earth system (Panikov et Sizova 2007). Permafrost hosts a potentially large pool of microorganisms, which is supposed to be the only life forms known to have retained viability over geological time. Thawing of the permafrost renews their physiological activity and exposes ancient life to modern ecosystems (Gilichinsky et al. 2008). The adaptation mechanisms of microorganisms, at species or population level, make them susceptible to extreme environmental condition. The survival of microorganisms in permafrost raises the question of what constitutes the limit for microbial life. Several studies of microbial populations have been conducted in Arctic or Antarctic permafrost (Guglielmin et al. 2005), but few studies concern the prokaryotic assemblage. Our contribution aims at filling some gaps in the knowledge about this peculiar and till now poorly studied microbial assemblage, evaluating the experimental methods to investigate it in terms of abundance, viability, ectoenzymatic activities and physiological profiles at community level.

METHODS AND MATERIALS

Six permafrost samples, collected in two sites of Northern Victoria Land: Boulder Clay (BC-FM, BC-S, BC-I and BC-5) and Edmonson Point (EP1), close to the Italian Antarctic station (MZS), and in Upper Victoria Valley within the McMurdo Dry Valleys (DY1) were analysed. Under sterile conditions and using a sterile trowel, material for microbial analyses was taken from the axial part of frozen cores and placed in sterile containers to be processed by standard techniques developed for sediments. To collect the associated microflora, all the samples were aseptically treated with two different extractions consisting of: 1) dilution in PBS and vortex homogenization; 2) addition of Tween 80 and sodium pyrophosphate, sonication and centrifugation. The following parameters were estimated on the two above referred extracts: prokaryotic cell abundance (Image-Analysis, IA; Flow-Cytometry, FC); viable and respiring cells quantification (Live/Dead and CTC+); potential rates of ectoenzymatic activities on proteinaceous (leucine-aminopeptidase, LAP) and glucidic (ß-glucosidase, ß-GLU) organic matter and on organic phosphates (Alkaline-fosfatase, AP); physiological profiles of microbial assemblage (Biolog-Ecoplate, BE).

RESULTS

Cell counts were in the range 10^6-10^7 cells ml^{-1}. Respiring cells (CTC+) accounted for the 1-5 % of total IA cell abundance only. Viable cells contributed from the 40 to 90 % of the total Live/Dead cells. Quantification of cell abundance and viable and respiring cells yielded to quite different results depending on the examined samples. FC evidenced several sub-populations (from one to four) with different apparent DNA content in the different samples. The utilization of carbon sources by BE showed that all the 31 available C sources were metabolized in the EP1 sample; 27 in the samples BC-FM, BC5 and DY1; only 21 and 15 in the BC-I and BC-S, respectively. With respect to the ectoenzymatic hydrolysis, the highest potential LAP and ß-GLU activity rates (0.491 and 2.925 µmol l^{-1} h^{-1}) were detected in the BC-I sample, while the maximum of AP activity (1.450 µmol l^{-1} h^{-1}) was measured in the EP1 sample.

DISCUSSION

The experimental study showed that different treatments are needed to investigate different microbial parameters. In terms of cell abundance, the highest IA and CTC+ enumerations were obtained by using extraction 1), whilst the highest CYTO and Live/Dead abundances by extraction 2). Regardless of the type of extraction used, in the sample BC-FM, the highest percentages of viable and respiring cells were observed. Permafrost hosted an enzymatically active microbial assemblage. BE outlined different physiological profiles at community level. This first screening of prokaryotic assemblage showed different patterns in each sample probably depending on the differences in permafrost conditions, age and lithology.

KEYWORDS: ANTARCTIC PERMAFROST; PROKARYOTIC ABUNDANCE; PROKARYOTIC METABOLISM; METHODOLOGIES.

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Poster B-12

INDUCTION OF MULTIPLE PROPHAGES FROM AN ANTARCTIC MARINE BACTERIUM

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INTRODUCTION

The genomic sequencing of the marine bacterium *Bizionia argentinensis (B.a)*, isolated from Antarctic surface water, has promoted the interest to know the functionality of their gene content. As part of this goal, we are studying the presence of a protein (that we called C24) showing structural homology with a T4 phage tail fiber protein. As it was previously reported that approximately 70% of sequenced bacterial genomes contain prophage-like structures, this finding of C24 suggested us the presence of genetic elements of the type of the prophages in the genome of *B.a.* Some prophages may contain morons or lysogenic conversion genes that could change the phenotype or enhance the ecological fitness of lysogens. Bacterial viruses represent one of three major mobile genetic elements and contribute significantly to horizontal gene transfer in bacterial genomes. However little is known about the characteristics, function and ecological relevance of marine phages. For this reason, in this work we search for the presence of prophages in a recently sequenced marine Antarctic bacterium.

METHODOLOGY

Induction of Ba with mitomycin C. The traditional and most common approach to studying prophages or temperate phages is to induce lysogenic bacteria with mitomycin C or UV exposure. We grown *B.a* overnight in marine broth incubated at 15°C in a rotatory shaker (200 rpm). Two ml of an exponential growth culture of *B.a* (optical density at 600 nm [OD600], ca. 1.0) was transferred to 50 ml of fresh marine broth (six replicates containing, 0.2 µg/ml mitomycin C and six without mitomycin C). After 24 hours, cells in both, control and treated groups, were pelleted by centrifugation (7,500g for 10 min) washed twice and resuspended in 50 ml of fresh marine broth. All cultures were incubated and monitored by OD600 at 8, 12, 24, 30 and 36 hours. Samples were kept at 4°C in the dark.

CsCl purification of induced phages. 50ml of induced viral lysate was centrifuged (10,000g, 4°C) and phage particles in the supernatant were purified using CsCl (as in protocol 8, Chapter 2.51, Maniatis). Phages particles were visulized by TEM after negative stain.

RESULTS

Fig. 1 shows the effect of Mitomycin C treatment on *B.a* growth. A clear deleterious effect, compatible with lysis by phages were observed.

TEM of the samples confirmed the presence of virused and denoted that at least three different phages were induced from mitomycin C treated cultures of *Bizionia argentinensis* (Fig. 2)

DISCUSSION

The biological effects of the presence of these multiple inducible phages in *Bizionia argentinensis* are still unknown, as also the genomic structure of them. Currently we are isolating the phages (lysis plaques formation) and subsequently its complete nucleotide genomic sequences will be done.

To our knowledge, this is the first work showing the induction of lysogenic bacteriophages from an Antarctic marine bacterium. Further studies about the abundance of lysogenic bacterial phages in the Antarctic environment and its biological relevance would provide a better view of the bacterial communities' dynamic of the coastal Antarctic marine environments.

KEYWORDS: LYSOGENIC, BACTERIAL, MYTOMICIN C, PROPHAGE, MORFOTYPE

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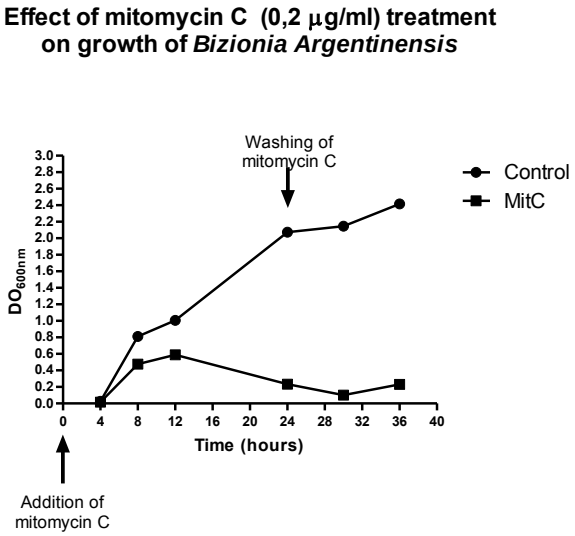

Fig. 1. Effect of mitomycin C on growth of *Bizionia argentinensis*. Cell densities were determined by measuring the optical densities at 600 nm in cultures treated with 2μg/ml mitomycin C for 24 hours and in control cultures without treatment.

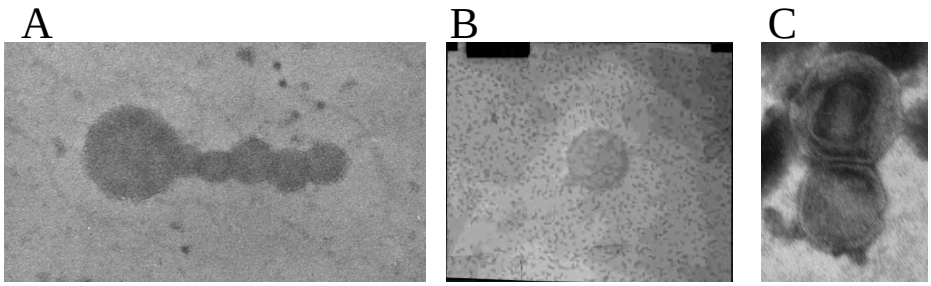

Fig. 2. Three viral morfotypes present in fractions 18-22 of the CsCl gradients from viral lysate supernatant (A, B and C).

Poster B-13

CONTROLLING FACTORS ON BACTERIAL DIVERSITY AND ACTIVITY OF COASTAL WATERS OF LAKE BAIKAL, SIBERIA

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Lake Baikal is the deepest freshwater lake located in permafrost zone. The coastal waters are firstly exposed by environmental and human factors. The goal of study is to determine the influence of environmental factors on the structural and functional bacterial changes of the coastal zone. We studied three sites of the eastern recreational coast of Lake Baikal in the different seasons 2012-2014. Multivariate dataset including the physicochemical (temperature, salinity, pH, redox potential, oxygen, bicarbonates, sulfates, chlorophyll a, nutrients) and microbiological (total number of bacteria, number of organotrophic bacteria, the intensity of production and degradation) parameters was analyzed by principal component analysis (PCA). It was found the most significant factors affecting the ecosystem are seasonal changes in the temperature and pH (28.5% of the observed changes). The second factor (16.7% of the observed changes) is location of the sampling (distance from the shore and depth), third factor (16.0% of the observed changes) is water salinity. Bacterial diversity were studied by next generation sequencing (NGS) established dominance of three bacterial phyla *Bacteroidetes, Proteobacteria, Actinobacteria*. The similarity of microbial diversity of coastal waters in three sites was found. Diversity of bacteria has seasonal clustering. We hypothesize that in the coastal zone bacterial diversity and activity are primarily driven by the seasonal temperatures.

KEYWORDS: CONTROLLING FACTORS, BACTERIAL DIVERSITY, COASTAL, LAKE BAIKAL

Poster B-14

PSYCHROPHILIC AND PSYCHROACTIVE BACTERIA IN COLD SPRINGS OF NORTHERN PRIBAIKALIE

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INTRODUCTION

The cold springs of Northern Pribaikalie (Republic of Buryatia, Russia) are characterized by low temperature (3-6°C), slightly alkaline pH reaction and oxidized conditions. Microbial communities of cold springs are the dominant life forms that involve in the process of matter and energy transformation in these ecosystems.

The aim of the investigation was to study the cultivated diversity of anaerobic psychrophilic and psychroactive bacteria in sediment samples of four cold springs Buksyhen located at the north-western side of Barguzin Depression.

METHODS AND MATERIALS

Cultivation of proteolytic bacteria carried by anaerobic method of cultivation (Hungate et al. 1969) on solid media at a temperature of 7°C. Analysis of the obtained bacterial strains was carried out by using method MALDI TOF mass spectrometry of whole cells.

RESULTS

The 24 strains of proteolytic anaerobic and facultative anaerobic psychrophilic and psychrotrophic bacteria were isolated from sediment samples. The isolated bacteria were divided into 7 groups by using the method of MALDI TOF mass spectrometry of whole cells.

The one representative of each group strains was selected and executed sequences of 16S rRNA genes with universal primers 27F. Phylogenetic analysis of 16S rRNA sequence by using GenBank database and program Blastn showed that closely related species for strain Buc-zh-1 was *Janthinobacterium lividum* with 99.4% similarity; strain Buc-p-16 was a closely related to species *Yersinia kristensenii* (99.1%) and strain Buc-ser-21 was related to species *Duganella zoogleoides* (97.3% similarity). These three strains are Gram-negative facultative anaerobic rods. Optimum temperature for growth was 29 °C for strain Buc-zh-1, 20 °C - for strain Buc-p-16 and 7-10 °C - for strain Buc-ser-21. Thus, the strains Buc-zh-1, Buc-p-16 and Buc-ser-21 were psychrotolerant, psychrophilic and obligate psychrophilic bacteria, respectively.

Also, we obtained pure cultures of psychrotrophic anaerobic hydrolytic bacteria with casein (10 strains) and xantan (2 strains) as carbon source. The microscopic investigation revealed rod-shaped bacteria differ in size, mobility and spore formation. Further analysis will allow characterizing and determining the taxonomic status of the isolated bacteria.

DISCUSSION

At the first time, pure cultures of psychrophilic and psychrotolerant proteolytic facultative anaerobic bacteria of the genera *Janthinobacterium, Yersinia* and *Duganella* were isolated from four Buksyhen springs, according to preliminary data, representing new species of taxa (Bercovier et al. 1980, Hiraishi et al. 1997, Schloss et al. 2010). Our study has shown that the psychrophilic and psychroactive hydrolytic microorganisms metabolic active at a low temperatures inhabit in the sediments of Buksyhen springs.

KEYWORDS: COLD SPRINGS, PSYCHROPHILIC BACTERIA, PSYCHROACTIVE BACTERIA, PHYLOGENETIC ANALYSIS.

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Poster B-15

HALOPHILIC AEROBIC MICROORGANISMS FROM ALYASKA CRYOPEG

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Many researchers have demonstrated the ability of pro and eukaryotic microorganisms to remain viable in permafrost, and specifically in cryopegs - lens of highly mineralized water existing at subzero temperatures within permafrost sediments of marine origin. Simultaneous exposure to permanent subzero temperature, highly salinity, background ionizing radiation over a geological time scale allowed us to consider this habitats as an extreme ecosystems to preserve an indigenous microbiota.

Recent microbiological investigations of cryopegs from the Kolyma lowland, Yamal and Varandey Peninsulas showed a significant diversity of viable cold-adapted organisms such as aerobic and anaerobic bacteria, archaea, yeast and filamentous fungi and viruses. Furthermore, some of halophilic psychrotrophic isolates were able to produce a cold-active lipolytic enzymes, which make these unique ecological niche a promising source of paleoorganisms and their metabolites for biotechnological applications.

This study is an initial step in describing biodiversity and biotechnological potential of the indigenous aerobic halophilic microbial community in the Alyaska cryopeg (Cape Barrow, NaCl – 100g/l). Diversity of cultivated aerobic halophilic bacteria were obtained by enrichment culturing on standard nutrient medium with wide range of NaCl concentration (5, 10, 50, 100, 150, 200, 250, 300 g/l) at 4 and 20°C.

Work bacterial collection (about 80 strains) was formed. Mostly salt-tolerant isolates were phylogenetically closely related to strains from different cold aquatic and terrestrial ecosystems (16S rDNA sequence similarities >98-99%) and belongs to phyla Proteobacteria (genus *Ochrobactrum*), Firmicutes (genera *Bacillus*, *Paenibacillus*, *Lacticigenium*), Actinobacteria (genera *Microbacterium, Brevibacterium, Citricoccus, Dietzia, Arthrobacter*) and Bacteroidetes (genus *Parapedobacter*). Members of phylum Firmicutes were able to grow at 4°C and high NaCl concentration – 300g/l, which is definitely important for biotechnology.

KEYWORDS: PERMAFROST, CRYOPEG, HALOPHILIC AEROBIC MICROORGANISMS, COLD-ACTIVE ENZYMES.

Poster B-16

MICROBIAL DIVERSITY AND COMMUNITY COMPOSITION ACROSS DEPTH GRADIENT IN MARINE SEDIMENTS FROM ADMIRALTY BAY, KING GEORGE ISLAND AND BRANSFIELD STRAIT, ANTARCTICA.

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Marine microorganisms play a critical role in ecosystem functioning and are the main drivers of the biogeochemical cycling of elements. They dominate in abundance and diversity, being responsible for the majority of the metabolic activity of the oceans (Azam et Malfatti 2007). This project aims to describe the microbial diversity across a depth gradient in marine sediments from Admiralty Bay (AMSA No.1), King George Island to the entrance of this bay at Bransfield Strait by molecular techniques and next-generation sequencing. Samples were collected from different locations of the Admiralty bay, with depths ranging from 100-1100 meters below sea level, during the XXVIII Brazilian Antarctic Operation. Total DNA from 0.3 g of each sediment sample was extracted using FastDNA Spin Kit for Soil (MP Bio Laboratories) and amplified with PCR specific primers targeting 16S rRNA gene. The Bacterial and Archaeal community profiles were analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) using primers 338GCF-518R and 344GCF-915R, respectively. The microbial community structure was analyzed by 454 pyrosequencing with barcoded universal primers 519F-1068R that corresponds to the V4-V6 regions of *E. coli* 16S rRNA gene. DGGE band profiles were compared in BioNumerics v 5.0, and ~117,000 tag-sequences were analyzed in Mothur v1.31. DGGE results revealed similar patterns for archaeal and bacterial domains, showing a difference between the shallowest samples (100, 300, 500 mbsl) and the deepest samples (700 and 1100 mbsl). General results obtained by 454 sequencing showed a high dominance of Gammaproteobacteria (89,3% mean abundance), followed by Alphaproteobacteria (3,7%), Firmicutes (1,5%), Bacteroidetes (1,3%), Deltaproteobacteria (1,3%) and Actinobacteria (1,0%). Archaeal sequences counted for less than 1% in all samples. Sequences similar to *Psychrobacter* (>98% similarity) were the most abundant among all sediment samples. *Psychrobacter* is a cold-adapted and chemoheterotrophic bacteria primarily isolated from cold to warm, slightly to highly saline ecosystems, including glacial ice, sea-ice, polar soils and marine sediments. Moreover, *Psychrobacter* was found associated to Antarctic macroalgae. The high abundance of a heterotrophic microorganism over 300 mbsl suggests a relatively high concentration of organic matter in the deep Antarctic marine sediment. Overall, these results indicate a homogeneus and heterotrophic environment in both shallow and deep marine sediment studied that could be explained by a high deposition of organic matter from macroalgae decomposition.

KEYWORDS: ANTARCTIC MARINE SEDIMENTS, KING GEORGE ISLAND, BIODIVERSITY, PYROSEQUENCING

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Poster B-17

MARINE RNA VIRUS COMMUNITIES OF OCEANIC SEAWATERS IN THE VICINITY OF THE ANTARCTIC PENINSULA

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Viruses play an important role in the biogeochemical cycle and the gene flux in marine plankton ecosystems. Multiple lines of evidence that RNA viruses comprise a significant fraction of virioplakton are emerging. However, no data on oceanic RNA viromes in the Antarctic seas are available. During the IBRV Araon cruise in 2013, each 50 L of surface seawaters at 4 offshore stations was collected and filtered to concentrate viruses by the $FeCl_3$ flocculation method. Marine viruses were recovered and purified by the CsCl gradient ultracentrifugation. Nucleic acids of viral fractions were extracted and subsequently DNAs were removed by adding DNase. Metagenome libraries were generated from RNAs using the random priming-mediated sequence-independent single-primer amplification. Size ranges of 300-600 bp in the libraries were selected and sequenced by 454 pyrosequencing with the GS-FLX Titanium chemistry, yielding 333 Mb with 764,764 reads as raw data in total. In the presentation, results and its implications will be discussed.

KEYWORDS: RNA, VIRUS, METAGENOME, ANTARCTIC, SEAWATER

Poster B-18

HOW UNIQUE ARE THE MICROMETAZOA OF ANTARCTIC SOILS? THE EXAMPLE OF BDELLOID ROTIFERS

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Bdelloid rotifers are one of the key groups of invertebrates populating the extremely stressful and isolated terrestrial and freshwater habitats of Antarctica (Adams et al. 2006). We studied the most up to date biodiversity, bio- and phylogeography, and ecology of Antarctic Bdelloidea, using both classical and molecular taxonomic approaches.

Our survey is based on both published data and large new collections made available from maritime Antarctica and the Ross Sea sector. The molecular diversity and distribution patterns of the four bdelloid genera occurring were analyzed using COX1 gene sequences. Species delimitation was based on both morphology and Bayesian phylogenies using three different approaches for taxon delimitation: 4x rule, Generalized Mixed Yule Coalescent (GMYC) and Poisson Tree Processes with Bayesian support (bPTP). Phylogeographic patterns were estimated using haplotype networks, and Nested Haplotype Tree Analysis of Geographical Distances (NHT).

The total number of bdelloid morphospecies previously known to occur in Antarctica and the sub-Antarctic was 49**.** From our collections we identified 60 morphospecies, of which 12 are new for science, and 5 are new for Antarctica (*H. angusticollis*, *M. nana*, *Mniobia incrassata*, *Mn. scabrosa*, and *Pleuretra lineata*). That extended the total list of Antarctic rotifers to 66 morphospecies (49 reported in the existing literature, and 17 newly found ones). The diversity estimated by molecular methods is 83-91 putative species (depending on the delimitation method used). Some of the "cryptic" species identified subsequently proved to be identifiable using high-resolution morphometrics, however at least three species remained recognizable by molecular methods alone. Only 13 of the morphospecies found occurred both in maritime and continental Antarctica. Only two as yet undescribed putative species, both within the genus *Philodina*, proved to be truly cosmopolitan, with the remainder being found only in Antarctica and/or the sub-Antarctic. The level of apparent endemism in Antarctic bdelloids based on the available dataset (95%) is higher than that of any other continent. NHT of *Adineta grandis* sampled along Victoria Land has shown association of genetic variation among haplotypes with geographical distribution, suggesting a pattern of long distance colonization (possibly from Beaufort and Ross islands) with subsequent local differentiation.

Our findings are consistent with and further strengthen the conclusions of recent studies of other Antarctic microscopic biota, pointing to long-term presence in Antarctica, considerable isolation and intra-regional radiation.

KEYWORDS: BDELLOIDEA, BIODIVERSITY, PHYLOGEOGRAPHY, DNA TAXONOMY

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Poster B-19

DIVERSITY OF CULTURED ICE CAVE MICROCOSM

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Ice cave represent an important environment for paleoclimate and exobiology studies. Considering the very limited data on the microbial diversity from this type of habitat, we are interested in characterizing the prokaryotic communities from cave ice layers of up to 2000 different aged from Scarisoara Ice Cave, Romania. Ice samples of 1, 400, 900, 1500 and 2000 years BP were collected from the cave ice block by vertical drilling. After cultivation on R2A medium at 4°C and 15°C using serial dilutions, the microbial cell density from ice strata ranged in the 10^2-10^4 CFU mL^{-1}, decreasing exponentially with the age of ice substrate. The morphology of dominant colonies from each sample was analyzed by optical microscopy and SEM, and their 16S rDNA was amplified and sequenced. Cultivation on different C-sources using BIOLOG EcoPlates system indicated similar functional diversity of 1 and 400 years old ice microbiota, strongly dependent on the conductivity, and total organic carbon (TOC) and total nitrogen (TN) contents of corresponding ice samples. Bacterial 16S rDNA OTUs were determined by PCR-DGGE analysis and sequencing of cultured ice samples. Individual and common bacterial OTUs of the analyzed cave ice layers belonged to *Gammaproteobacteria, Firmicutes, Bacteroidetes*, and *Actinobacteria* representatives reported in various cold environments and karst habitats. While recent sun exposed ice microbiota was dominated by *Gammaproteobacteria*, *Firmicutes* occupied the major phylum of 400 years old ice, and both *Bacteroidetes* and *Gammaproteobacteria* showed the highest relative abundance in 900 years old ice. A high number (28%) of unclassified bacteria suggested a broader diversity of cave ice microcosm. 16S DNA sequence identification of dominant colonies obtained at low and high temperatures on R2A from the 1, 400, 900, 1500 and 2000 years old ice samples is currently underway. This survey on the abundance, distribution and diversity of bacterial communities embedded in the perennial ice block of Scarisoara Ice Cave emphasizes the potential of this glacial ecosystem to identify microbial climate biomarkers.

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KEYWORDS: ICE CAVE, CULTURED BACTERIA, 16S rDNA, DIVERSITY

Poster B-21

EPI- AND ENDOPHYTIC MICROBIAL COMMUNITIES OF ARCTIC AND SUBARCTIC PEATLAND MOSSES

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INTRODUCTION

Northern peatlands are one of the major natural sources of the greenhouse gas methane. Bryophytes such as *Sphagnum* and *Amblystegiaceae*, the so called brown mosses, are major constituents of the plant community of those peatlands and can be associated with microbial communities of the methane cycle. While *Sphagnum* mosses thrive in oligotrophic, acidic bogs, members of *Amblystegiaceae* grow in mesotrophic, neutral wetlands. Most studies were concerned with *Sphagnum* and their associated methane oxidizing bacteria, and especially submerged habits of *Sphagnum* showed high methane oxidation activities (Kip et al. 2010). Microorganisms associated with brown mosses were so far largely neglected, despite their considerable contribution to reduce methane emissions e.g. from Arctic polygonal peatlands (Liebner et al. 2011). Methane producing archaea in association with mosses of northern peatlands were never investigated so far, although they directly contribute to global methane emissions and their association with peatland mosses was observed in pilot experiments.

METHODS AND MATERIALS

We sampled a variety of *Amblystegiaceae* and *Sphagnum* species from altogether 26 sites in Svalbard (Spitsbergen), Samoylov Island (Lena Delta, Siberia), Finnmark (Northern Norway) and temperate peatlands (Serrahn, Northern Germany). Additionally reference samples (sedges, sediment) were obtained from all sites. To distinguish between epiphytic and endophytic microorganisms, we adapted a protocol based on ultrasonic cleaning prior to surface sterilization. Epi- and endophytic bacterial and archaeal communities of mosses were analyzed by 454 sequencing of 16S rRNA gene. Cell wall analysis (lignin, holocellulose, CEC) and C/N determination of mosses and sedges were carried out and environmental parameters (pH regime, DOC, organic acids) as well as temperature, CH_4 and O_2 gradients in pore waters were measured and used for statistical analysis.

RESULTS

We succeeded to separate epi- from endophytic microbial communities of both, *Sphagnum* and brown moss species. More versatile microbial communities occur in brown mosses of neutral, mesotrophic wetlands than associated with *Sphagnum*. Microbial colonization also seems to be more pronounced in inundated sites. The differences between epi- and endophytic microbial communities where little while geographic location and plant taxa shaped the structure of both, bacterial and archaeal communities.

DISCUSSION

For the first time we show the community structure of epi- and endophytic bacteria and archaea associated with *Sphagnum* and brown moss species and their environmental controls. It is known that environmental parameter like pH and water level as well as biogeography shape microbial communities, which is also confirmed here.

Our data raise the question if moss-microbial-associations are rather influenced by moss species or environmental parameters.

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Poster B-23

TRANSPORTATION AND PERSISTENCE OF MICROBIAL CELLS IN CENTRAL WEST ANTARCTICA

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Very few studies have been conducted on the transportation and persistence of microorganisms into the Central West Antarctica. The source of potential airborne microbiota is widespread, as they may originate from water surfaces, soils, plant surfaces, animals, and clouds. So much of what we know about the probable dispersion and behavior of airborne microbiota comes from studies undertaken in other parts of the world. The research launched in the Criosfera 1 remote lab (a scientific module installed in the summer of 2011/2012 at 84°S and 79°30'W by the Brazilian National Institute of Technology for the Cryosphere) is trying to help to understand how microorganism are transported to the West sector of Antarctica and how they persist in the oligotrophic and freezing snow environment. Atmospheric transport models as the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) suggest that Criosfera 1 location receives major influences of air masses originated from both the Weddell and Bellingshausen sea sectors and from the Antarctic Plateau. Snow samples collected from a 2 m deep pit dug at Criosfera 1 area in 2014/2015 austral summer are being analyzed by microscopy, cultivation-based and DNA-based methods to investigate the presence of microorganisms in the region. Cell count from samples collected each 20 cm in depth indicated a cell abundance of 0.8 - 1.5 X 10^2 cells mL^{-1}, one to two orders of magnitude lower than the cells abundance found in the South Pole and other Antarctic continental areas. Epifluorescence microscopy analyses indicated a considerable diversity in cellular shapes ranging from ultra-small cells with diameter of 0.2 µm to rod shape cells up to 1.3 µm in length. Up to date, cell culturing and phylogenetic analyses from the total DNA extracted from the snow samples are in development to identify the microbial population existing in the local under investigation. We expect that by conducting simultaneous analyses among atmospheric modeling pathways, isotopical and elemental determination, and the microbial populations existent in the samples, we will be able to infer about the major atmospheric input of biological cells associated to inorganic microparticles in the West Antarctica within the past decades and infer about the mechanisms used by these cells to persist in this extremely cold environment.

KEYWORDS: AIRBORNE MICROBIOTA, CRIOSFERA 1, CENTRAL WEST ANTARCTICA

Poster B-24

BIOGEOGRAPHICAL DIVERSITY OF ROOT ASSOCIATED MICROBIAL COMMUNITIES IN ARCTIC AND ALPINE TUNDRA PLANTS

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We are interested in bio-geographical diversity and functioning of plant associated microbial communities in the Arctic and Alpine tundra plants. In this study we addressed community composition of soil, rhizosphere and root endophytic bacterial and fungal communities associated with two arcto-alpine plant species, *Oxyria digyna* and *Saxifraga oppositifolia* in three climatic regions. The samples were collected from Kilpisjärvi, Finland (sub-arctic), Ny-Alesund, Svalbard (high arctic) and Innsbruck, Austria (alpine). Terminal restriction fragment analysis (T-RFLP) and massive parallel sequencing were used to address the influence of location, climate and plant species on structure of soil and plant microbial communities.

Community fingerprinting analysis of bulk and rhizosphere soils revealed that soil bacterial communities were primarily shaped by climate, geographical location and sampling sites. Within the sampling sites the influence of individual plant species on rhizosphere community composition was clear, suggesting that plant species select their own rhizosphere bacterial community from available local soil microflora. A similar trend was detectable in the community fingerprinting and sequence data of fungal communities of bulk soil and rhizosphere soil communities from different climatic zones. In contrast, the community structures of root endophytic fungi were primarily influenced by plant species, and only secondarily by climatic zones, soil properties or geographical distance. Sequence analysis of soil and rhizosphere fungal communities indicated an increase in phylum Zygomycota and decrease in phylum Chytridiomycota along the latitude from Austria, Kilpisjärvi to Ny-Alesund, while no such trend was observed in other phyla. In addition, we observed that *O. digyna* and *S. oppositifolia* had several plant species specific root endophytic fungal OTUs present across all the regions. These host plant specific fungal species were absent in both bulk and rhizosphere soils.

Poster B-25

POLAR AND ALPINE MICROBICAL COLLECTION (PAMC): A CULTURE COLLECTION DEDICATED TO POLAR AND ALPINE MICROORGANISMS

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The number of microbial strains isolated from polar and alpine areas is increasing and they are recognized as valuable resources in fundamental studies, such as ecology, physiology, and –omics. Thus, the necessity of culture collection dedicated to the polar and alpine microorganisms has increased. Korea Polar Research Institute (KOPRI) established the Polar and Alpine Microbial Collection (PAMC) to share biodiversity information and bio-resources collected from polar and alpine areas in science and public communities. Approximately 2,500 out of 7,500 strains maintained in PAMC have been identified and belonged primarily to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Many of the microbial strains of PAMC can grow at low temperature and produce proteases, lipases, and/or exopolysaccharides. PAMC provides search tools based on keywords such as taxonomy, geographical origin, habitat and physiological characteristics. Biological materials and information provided by PAMC will be important resources for those who have had no opportunity to visit polar and alpine areas and are expected to contribute to the development in the extreme life sciences.

KEYWORDS: BIODIVERSITY, MICROORGANISMS, PHYSIOLOGICAL CHARACTERISTICS

Poster B-26

GENOMIC AND PHENOTYPIC CHARACTERISTICS OF A THERMOPHILIC *Bacillus* sp. 9F ISOLATED FROM DEEP-SEA HYDROTHERMAL VENT PLUME, SOUTHERN OCEAN

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INTRODUCTION

The strain 9F was isolated from deep-sea hydrothermal vent plume in Australian-Antarctic Ridge in the Southern Ocean. Analysis of the 16S rRNA gene sequence of strain 9F showed an affiliation to the type strain of *Bacillus lichneformis* with a high similarity of 99.9%. In the present study, we compared genomic and phenotypic features of strain 9F with those of the type strain of *B. lichneformis* (KCTC 1918^T) to find differences between the strains of different origins; strain 9F is the first strain from deep-sea hydrothermal vent environment, while other strains *B. licheniformis* have been isolated from diverse terrestrial environments including the type strain.

METHODS AND MATERIALS

A genome sequence of strain 9F was determined using an Illumina MiSeq, whereas a complete genome sequence of the type strain of *B. licheniformis* was retrieved from the GenBank under the accession AE017333 (4,222,645 bp with the G+C content of 46.2 mol%). Temperature and salinity ranges for growth were determined for both strains. Enzyme activities, carbon assimilation, acid production from carbohydrates were assayed using the API ZYM, 20NE and 50CH kits.

RESULTS

A draft genome of strain 9F revealed 29 contigs in 4,376,753 bp with the G+C content of 45.6 mol%. Genomic relatedness analyses based on average nucleotide identity and the genome-to-genome distance showed that strain 9F and *B. licheniformis* KCTC 1918^T belonged to single species. A lower limit of temperature for growth was 10°C and 15°C for strain 9F and *B. licheniformis* KCTC 1918^T, respectively, while an upper limit of temperature was 60°C for both strains. Salinity tolerant tests showed an identical result for both strains (0–7.5% NaCl, w/v). No difference between both strains was found in the enzyme profiles with the API ZYM and 20 NE assays. However, an obvious difference in acid production (14 out of 49 carbohydrates in the API 50CH assay) and the assimilation of arabinose were observed between them.

DISCUSSION

Preliminary results of comparative genomics between strain 9F and *B. licheniformis* KCTC 1918^T showed some fragments of DNA were either inserted or deleted in one of the genomes. Interestingly, the different fragments were often attributed to viral DNAs, suggesting that a horizontal gene transfer mediated by phages might serve to shape phenotypic traits of their host bacteria originated from different habitats.

KEYWORDS: GENOME, THERMOPHILE, BACTERIA, VENT, SOUTHERN OCEAN

Poster B-27

STRATIFICATION OF MICROBIAL COMMUNITY IN MARINE SEDIMENTS OF THE ROSS SEA, ANTARCTICA

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The deep subseafloor biosphere is the least-understood habitat on Earth, even though the amount of microbial biomass therein plays important roles in the biogeochemical cycles and remineralization of organic materials. In this study, microbial community of 21 sediment horizons from one gravity core (approximately 4 m) collected in the Ross Sea was profiled by pyrosequencing. Distinct stratification in the microbial community within the gravity core was observed. Bacterial community showed distinctive stratification from *Proteobacteria, Planctomycetes, Bacteroidetes, Acidobacteria,* and *Chlorobi* in the aerobic upper sediment column towards OP9, *Chloroflexi*, and *Actinobacteria* in anaerobic sediment horizons. Interestingly, uncultured candidate phylum OP9 was predominant from 40 cm below seafloor composing upto 54.2%, indicating initial constraints for their microbial habitat preferences. Archaea also showed a dramatic shift in community composition at the oxic-anoxic transition zone as was the case for bacteria. *Crenarchaeota* was the most dominant archaeal phylum throughout the sediment. However, relative abundance of *Crenarchaeota* classes varied considerably along the depth. Eukaryotic community showed *Stramenopiles* and unassigned group to any phyla were dominant throughout the sediment. However, relative abundance of *Alveolata* and *Metazoa* showed significant decrease along sediment depth, especially across the oxic–anoxic transition.

KEYWORDS: DIVERSITY, SEDIMENT, ANTARCTICA

Poster B-28

BIO-MINING THE ACTINOBACTERIAL TREASURES OF THE ARCTIC OCEAN: DIVERSITY AND GENETIC RESOURCES

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Actinomycetes are an important group of bacteria with wide distribution and abilities to producing bioactive compounds such as well-known antibiotics. However, diversity of actinomycetes in the remote Arctic Ocean and their abilities to produce bioactive products have seldom been studied systematically. To improve our understanding of actinomycetes inhabiting the Arctic marine sediments, both culture-independent and -dependent approaches were used to reveal the diversity. In addition, natural products were primarily investigated for representative isolates using HPLC-MS. Target genome was sequenced to explore genetic resources as well as to investigate its metabolic diversity. In our investigation, actinomycetes accounted for around 10% of the bacterial communities. Highly diverse actinobacterial communities were found inhabiting the Arctic marine sediments, revealed by Miseq sequencing. A total of 298 actinomycete strains were isolated, grouped into 18 genera including *Streptomyces*, *Nocardiopsis* and *Rhodococcus* as the three most dominant genera. Complex natural products were potentially produced by the tested isolates. New natural products were expected according to the primary HPLC-MS analysis. The genome sequencing of a representative strain, *Streptomyces* sp. 604F, revealed 28 gene clusters potentially encoding secondary metabolites. Some of the gene clusters were predicted to be cryptic, and possibly producing new natural products. In addition, a large portion of the genes predicted in the genome was new with unknown function or no similarity with known genes. In summary, we found diverse actinobacterial communities inhabiting the Arctic marine sediments, and isolated actinomycetes with potential in producing various natural products. Even larger genetic capacity was shown to produce diverse secondary metabolites, indicated by genome mining. It suggests that more unknown microbial treasures than we previously expected are to be found in the Arctic Ocean.

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KEYWORDS: ARCTIC, ACTINOMYCETES, GENOME, DIVERSITY

Poster B-29

MICROBIAL ABUNDANCE AND METHANOTROPHY IN DEGRADING SUBSEA PERMAFROST FROM THE LAPTEV SEA SHELF, SIBERIA

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INTRODUCTION

Subsea permafrost formation and its subsequent degradation result from the inundation of terrestrial permafrost during the Holocene marine transgression and (or) coastal retreat since the Last Glacial Maximum. Estimated methane release rates to the atmosphere from the Siberian Arctic shelf suggest pronounced (Shakhova et al. 2013) as well as negligible climate impacts (Overduin et al. in press). Given the large area of subsea permafrost estimated to be about 2.5 times the size of the yedoma region (Schuur et al. 2015) potential carbon loss such as in the form of released methane in either case is of concern. Microbial communities trigger the breakdown of carbon and the associated release of greenhouse gases. However, our knowledge on microbial abundances and carbon turnover in subsea permafrost is poor. We expect that ice bonded subsea permafrost preserved microbial communities and that sea water induced warming of permafrost for centuries and millennia even stimulated microbial growth and carbon turnover. In addition, methane that is released following thaw is likely to be oxidized anaerobically with sulfate as electron acceptor by so called anaerobic methanotrophs (ANMEs) creating a deep sulfate methane transition zone at the thaw front as suggested recently (Overduin et al. in press).

MATERIALS AND METHODS

We studied two subsea permafrost cores from the western and central Laptev Sea, C2 and BK2. The ice-bonded, primarily sandy deposits of both cores are of Pleistocene origin with similar hydrochemistry and temperature regime. C2 is located 11500 m offshore and has been flooded about 2500 years ago. BK2 is located only 800 m offshore and has been flooded about 540 years ago. We applied SybrGreen staining along the submarine permafrost units to estimate total microbial cell counts (TCC) and compared these data with cell counts of the overlain unfrozen sediments. In order to analyze microbial processes at the permafrost thaw front we used fluorescence in situ hybridization (FISH), quantitative PCR and 16S rRNA amplicon sequencing to target overall archaea and bacteria as well as ANME communities.

RESULTS

TCC along the subsea permafrost units varied by more than three orders of magnitude ranging from 1.6×10^4 to 4.6×10^7cells g^{-1} sediment. In C2, they were on average two orders of magnitude higher than in BK2 and exceeded TCC of the overlain unfrozen sediments two to three times. At the thaw front of BK2 we observed a two to three fold increase of bacterial and archaeal cell numbers when compared with samples directly above and below. Using FISH, the existence of ANMEs, though indicated through SybrGreen stained microbial consortia, could not be verified as a result of low microbial activity. However, amplicon-based molecular analyses point at their existence.

DISCUSSION

TCC are comparable with those of various subseafloor sediments at similar depths (Kallmeyer et al., 2012) with the tendency towards relatively high values in C2. The significantly higher amount of cells in C2 compared with BK2 may be due to a longer period of permafrost warming being supportive for the resident community in C2. This, however, is pure speculation. Our data on TCC from subsea permafrost are unique. They prove that terrestrial derived permafrost is a source of deep submarine life with areas of comparatively high microbial abundance. Furthermore, they exposed large heterogeneities between subsea permafrost deposits despite environmental and evolutionary similarities and point out the need for further microbial studies in this field. Our results also suggest stimulated but low microbial activity at the permafrost thaw front most likely involving the anaerobic oxidation of methane through ANME members. Low methanotrophic activity was to be expected given slow liberation rates of methane of ~120 mg CH_4 m^{-2} y^{-1}.

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Poster B-30

DIVERSITY AND ECOLOGY OF MICROORGANISMS FROM COLD SEEPS OF NATIONAL PARK "ALKHANAI" (TRANSBAIKALIA, RUSSIA)

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Diversity, abundance, and the distribution of microorganisms in the extreme environments are determined by the efficiency of their adaptive mechanisms (Yumoto 2013, etc.). Natural underground waters are extreme ecosystems for life. The main differences them from surface water ecosystems deals with the total or partial isolation from the inflow of organic matter and energy (Chivian et al. 2008). The aim of our study was to investigate the diversity of microorganisms from the cold ultra-fresh oligotrophic seeps of the National Park "Alkhanai".

National Park "Alkhanai" is located in the Duldurginskij region of Transbaikalia, Russia. The main paleovolcanoe is Mount Alkhanai with a height of 1662 m. The thickness of the zone hydrogeological effective fracture consists of 60–100 m, and depth to groundwater up to 1.5–3.0 m. Aquitard for the complex is long-term and seasonal frost. Within Alkhanayskij massif in the most mountainous part, on the area of 21.5 km^2, 14 springs with a total flow rate of 89.3 L/s have been found, whereas at the foot of the Mount Alkhanai individual springs' flow rate consists of 10–15 L/s. These springs are sodium, bicarbonate-calcium, mixed according to water chemistry. Waters are ultra-fresh with total mineralisation of 50.42 mg/L and pH of 5.9–6.6.

Water samples were collected from the six seeps (Seeps 1 and 2, and Eye, Cardiac, Gastric, and Kidney Arshans) and River Sukhoe Ubzhogoe at the exit point to the surface in August 2009 and July 2010 for microorganisms' cultivation, microscopic and molecular analysis. Physico-chemical characteristics of water (pH, salinity, dissolved oxygen, and temperature) were determined using a field laboratory Multi-340i (WTW, Germany). Functional activity of microbial communities was determined by the radioisotope method (Sorokin 1975).

The intensity of microbial processes in the cold seeps of Alkhanaj National Park was comparable with the processes in the waters of oligotrophic lakes. The dark CO_2 assimilation is mostly carried out by chemoorganoheterotrophic bacteria (about 90%), consists of 0.453–0.594 mkg C ml^{-3} day^{-1}, which indicates preferential development of heterotrophic microbial community in investigated waters. In the seeps sediments sulfate reduction prevailed over methanogenesis on the terminal stage of organic matter decomposition that distinguishes these seeps from other freshwater ecosystems and may be explained by the percolation of deep cold fluids.

Microscopic examination revealed that different origins of the groundwater formation and the special conditions of groundwater release to the surface have affected the structure of microbial communities (Table). Cultivation has shown that chemoorganoheterotrophic bacteria represented a relatively small fraction (0.005–0.06%) of the TBN in the water of the River S. Ubzhogoe. Low values of the number of cultivated microorganisms (NCM, Table) were observed in the water of Seeps 1 and 2, Gastric Arshan, and River S. Ubzhogoe; they corresponded to the oligotrophic level and microorganism distribution in the freshwater river ecosystems. The maximum values of the indicator were found in the Cardiac and Kidney Arshans (Table). The results of the quantitative enumeration of pigmented chemoorganoheterotrophic microorganisms in the studied waters have established an unusually high amount varied from 0.1 to 90% of the microbial community.

Comparative analysis of 16S rRNA gene fragments of pure cultures and native samples showed a low diversity of bacteria and revealed three major phyla of Eubacteria: Proteobacteria, Bacteroidetes, and Actinobacteria. Phylogenetic analysis revealed that the red-pigmented bacteria were identified as *Serratia* spp., purple-pigmented as *Duganella* sp. and *Janthinobacterium* sp., and yellow-pigmented as *Pseudomonas* spp. The role of pigments in the bacterial life and metabolism is currently not fully understood. Thus, research on natural extreme environments may be promising for the elucidation of the role of these pigments in the bacterial life.

KEYWORDS: COLD SEEPS, HETEROTROPHIC MICROORGANISMS, DIVERSITY, ALKHANAI

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Table. Physico-chemical and microbiological characteristics of cold ultra-fresh oligotrophic seeps and River S. Ubzhogoe.

Name of sampling site	Seep 1	Seep 2	Springhead of the River Sukhoe Ubzhogoe	Upstream of the River Sukhoe Ubzhogoe	River Sukhoe Ubzhogoe			
					Eye Arshan	Cardiac Arshan	Gastric Arshan	Kidney Arshan
Temperature, °C	0.6	1.5	1.3	2.7	2.7	2.7	2.9	2.8
pH	6.5	6.4	6.3	6.3	6.7	6.5	6.7	6.7
Mineralisation, mS/sm	28	50	36	37	31	52	54	53
Oxygen, mg/L / % of saturation	7.60/66.6	7.51/90.4	7.85/92.8	7.40/64.3	7.40/64.3	7.40/64.3	7.40/64.3	7.40/64.3
Total bacteria number (TBN), $\times 10^6$ cells/mL	0.06	0.15	3.73	2.33	1.98	3.01	0.78	3.45
Rod size (h×d), um	2.0–4.0 ×1.0–2.0	2.0–4.0 ×0.6–1.5	1.5–3.0 ×0.8–1.5	2.0–4.0 ×1.0–1.5	0.8–6.0 ×0.4–1.6	0.8–6.0 ×0.4–1.6	0.8–6.0 ×0.4–1.6	0.8–6.0 ×0.4–1.6
Cocci size, d, um	0.8–1.0	1.0–1.5	1.0–1.5	1.0–1.5	1.0–1.5	1.0–1.5	1.0–1.5	1.0–1.5
Biomass, mg/L	0.36	0.45	14.29	11.69	8.69	7.8	0.9	8.33
Number of cultivated microorganisms (NCM), $\times 10^3$ CFU/mL	0.7	0.07	0.22	1.5	1.53	3.5	0.91	4
Number of morphotypes of cultivated microorganisms	3	4	3	4	4	4	4	4
Fraction of NCM from TBN, %	1.16	0.04	0.005	0.06	0.07	0.12	0.12	0.11

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Poster B-31

THE SEARCH OF METHANOGENS IN ARCTIC AND ANTARCTIC PERMAFROST

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Over the past few decades, researches have shown that low-temperature ecosystems play an important role in shaping the Earth's climate and the balance of greenhouse gases in the atmosphere, and the tundra zone is a significant source of biogenic methane. In the nineties, it was established that in addition to the seasonally thawed layer, a significant amount of methane was in permafrost grounds, which never thawed after freezing, and currently, this methane is derived from the modern biogeochemical carbon cycle. Later, the experiments with radioactively labeled substrates showed that methanogenesis can occur in samples of permafrost deposits at subzero temperatures up to -16.5^{o}C (Rivkina et al. 2004). These results have demonstrated the possibility of the metabolic activity of methanogens in permafrost ground. Subsequent studies of Pliocene and Pleistocene age permafrost using classical microbiological methods allowed to identify and to describe methanogenic archaea of *Methanosarcina* and *Methanobacterium* genera (Rivkina et al. 2007). Also the strain of *Methanosarcina soligelidi*, isolated from permafrost-affected soil was described (Wagner et al. 2013). All methanogens isolated from Arctic permafrost ecosystems were mesophiles.

The purpose of this study was to try to identify and isolate new taxa of methanogenic archaea capable of growing at low temperatures in Arctic and Antarctic permafrost samples. Based on obtained metagenomic data we analyzed techniques and media for isolation of methanogens (Krivushin et al. 2015).

Experiments with low temperature preincubation effect, gas phase content and using different substrates for stimulation of methanogenesis in Arctic samples of different age were conducted. Obtained results showed preincubation to have success for one week at 4^{o}C and gas phase consisting of CO_2.

At the studying of the methane accumulation dynamics in the anaerobic enrichment cultures we repeatedly noticed the alternation of the methane formation and oxidation that can testify to presence of anaerobic methanotrophs in the Antarctic permanently frozen grounds. The results of our investigations helped us to suggest the potentially assistance of cyanobacteria in the anaerobic methane oxidation process.

KEYWORDS: METHANE, METHANOGENS, PERMAFROST

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Poster B-32

PROKARYOTIC COMMUNITY STRUCTURE ACROSS THE ICE BLOCK OF SCARISOARA CAVE DETERMINED BY 454 PYROSEQUENCING

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One of the largest and oldest cave ice block hosted in Scarisoara Ice Cave, Romania, revealed the presence of cultured microorganisms in clear and organic-rich ice strata (Hillebrand-Voiculescu *et al*. 2015). The prokaryotic diversity was assessed by 454 pyrosequencing of 16S rRNA encoding gene pool of microbial communities from various locations of the ice block. Five samples containing clear and organic-rich ice strata of 1, 400 and 900 cal. yrs. BP were collected in triplicate from dark and light-exposed areas, and a comparison of their bacterial and archaeal 16S reconstituted diversity was carried out based on the 273,000 reads and 14,000 species resulted. Total and viable cell abundance of ice-embedded microbiota was determined by flow cytometry. The organic carbon and nitrogen contents of the ice samples were measured and correlated with the microbial density and diversity from the investigated cave ice deposits. The dominant bacterial phyla showed variations with both the age and C/N content of the ice substrate. Archaeal phylotypes were identified only in 400 and 900 years old ice, the dominance of their autotrophic/heterotrophic metabolism being dependent on the organic content of ice substrate. The current study, representing the first overview of the microbial diversity in a cave ice block using 16S gene pyrosequencing, could be used for assessing the impact of climatic and environmental changes recorded up to at least 5,000 years in this alpine ice cave.

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KEYWORDS: ICE CAVE, 16S rRNA GENE, PYROSEQUENCING, DIVERSITY

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Poster B-33

COMMUNITY COMPOSITION, DIVERSITY AND ACTIVITY OF N FIXING CYANOBACTERIA ASSOCIATED WITH MOSSES IN SUB-ARCTIC ALPINE ECOSYSTEMS

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INTRODUCTION

Moss associated cyanobacterial communities (MAC) are thought to be major contributors to N input in high latitude regions. However, most studies have been carried out in the boreal forest (Rousk et al. 2013) and the High Arctic (Solheim and Zileke 2013

), whereas biological N_2 fixation in other moss-rich regions such as the sub-arctic may also be largely MAC-based. Our preliminary results suggest that (*i*) MAC contribute to the N budget of widespread moss-dominated terrestrial sub-arctic and alpine ecosystems, and (*ii*) in these ecosystems cyanobacteria show specificity in associating with different moss species. The aim of this study is to evaluate diversity, specificity, abundance and N fixation activity of cyanobacteria associated with four moss species: *Racomitrium lanuginosum*, *Hylocomium splendens*, *Pleurozium schreberi* and *Sanionia uncinata*, all abundant in moss-dominated sub-arctic ecosystems in Iceland.

MATERIAL AND METHODS

Moss samples were collected from a moss-heath dominated birch woodland on postglacial basaltic lava in SW Iceland (64°04’ N, 21°44’ W) and from two International Tundra Experiment (ITEX) sites, (*i*) a mesic dwarf birch heathland (65°13’ N, 19°42’ W, 450 m altitude) largely covered by mosses, and (*ii*) a *Racomitrium* moss heath on postglacial lava (64°17’ N, 21° 05' W, at 120 m). Cyanobacterial identification and quantification was carried out by phase-contrast, fluorescence and confocal scanning microscopy. Further estimation of the types and relative abundance of cyanobacteria was performed by amplification and sequencing of *nifH* genes. N fixation was assessed with the acetylene reduction assay (ARA).

RESULTS

The cyanobacterial strains identified appeared to be from the orders *Stigonematales* and *Nostocales*. Sequencing and phylogenetic analysis showed that *N. punctiforme* was the most common cyanobacterial species associated with the moss species uder study. The highest diversity of MAC was found in *R. lanuginosum.* N fixation varied over time, also it was responsive to microclimatic/micro-topographic gradients. Simulated climate warming and grazing negatively affected N fixation activity. Moss water content and type of vegetation were the most influent parameters on potential N fixation activity.

CONCLUSION

Our finding about MAC in moss-widespread sub-arctic ecosystems may have substantial impact on the understanding of the N cycle in this terrestrial environment

NEXT STEPS

In addition to direct microscopic counting, real-time quantitative PCR (qPCR) will be used in order to determine the relative abundance and dynamics of *nifH* genes, which act as proxies for diazotrophic cyanobacteria. The N fixation activity of MAC will be validated by uptake of isotope labelled nitrogen (^{15}N).

KEYWORDS: DIVERSITY N_2-FIXATION CYANOBACTERIA MOSSES SUB-ARCTIC

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Poster B-34

LICHENSPHERE: A PROTECTED NATURAL MICROHABITAT OF THE NON-LICHENIZED FUNGAL COMMUNITIES LIVING IN EXTREME ENVIRONMENTS OF ANTARCTICA

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Antarctica is the largest extreme continental region of the planet and the same time a unique field laboratory to study the diversity and ecology of life forms under extreme conditions. In the present study, we surveyed the diversity, distribution and ecology of non-lichenized fungal communities associated with the Antarctic lichens *Usnea antarctica* and *Usnea aurantiaco-atra* across the latitudinal gradient in Antarctica. The phylogenetic study of the 438 fungi isolates recovered revealed the identification of 58 taxa from 22 genera of *Ascomycota*, *Basidimycota* and *Zygomycota*. The most abundant taxa obtained were *Pseudogymnoascus* spp., *Thelebolus* spp., *Antarctomyces psychrotrophicus* and *Cryptococcus victorie*, which are considered endemic and/or highly adapted fungi in the Antarctic environments. Thirty-five fungi may represent new and/or endemic species, including the *Pseudogymnoascus* and *Thelebolus* taxa. The fungal communities associated with Antarctic lichens displayed high diversity, richness and dominance indices; however, the similarity of the fungal communities was variable among the *Usnea* species as well as across the Antarctic islands. After discover an unexpected rich and diverse non-lichenized fungal communities associated with the thalli of the Antarctic lichens, composed by symbionts, decomposers, parasites, endemic and cold-adapted cosmopolitans taxa, we introduced the term “lichensphere”. We hypothesized that the lichensphere may represent a protected natural microhabitat with favourable conditions able to help non-lichenized fungi and other Antarctic life forms survive and disperse in the extreme environments of Antarctica.

KEYWORDS: FUNGI, ANTARCTIC LICHENS, DIVERSITY.

Poster B-35

AERIAL TRANSPORT OF BACTERIAL CELLS IN THE ARCTIC: SOURCES, DEPOSITION AND IMPACT ON ATMOSPHERIC PROCESSES

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Due to poorly constrained atmospheric concentrations of ice nucleating particles in the arctic, it is difficult to estimate the radiative energy budged of this region. Our study aims at identifying the sources, concentrations, and the distribution of active airborne bacterial cells that may be involved in cloud development and ice crystal formation, and are thus among the drivers of meteorological and climatic processes in the Arctic. It is known that a substantial proportion of bacterial cells maintain viability shortly after their emission from ground to the atmospheric boundary layer (72%-95% of all cells were viable, Hill et al. 2007), after their transfer to the free troposphere (60%-100%, DeLeon-Rodriguez et al. 2013) and even after long-range transport (16%-40%, Hara and Zhang 2012). There is also increasing evidence that airborne bacteria interact with cloud droplets and initiate ice formation, by which they impact cloud and precipitation development (Christner et al. 2008, Šantl-Temkiv et al. 2015). Nonetheless, the *in situ* general- and ice-nucleation-activity of airborne bacteria has yet to be confirmed.
We collected over 50 air, snow, and rain samples at Nuuk Basic (southwest Greenland) and at Station Nord (northeast Greenland) in order to study local and distant sources of airborne bacteria as well as their potential impact on atmospheric processes in the Arctic. Airborne bacteria were captured and up-concentrated in an RNA-preservation liquid using a high-volume impinger. At Nuuk Basic, samples of dominant plant species, rock surfaces, exposed soil, and fjord and lake water were collected to investigate local sources of airborne bacterial cells. At Station Nord, snow samples were collected in order to study deposition of bacterial cells after long-range transport. SSU rRNA and SSU rRNA genes were quantified by (RT-)q-PCR to enumerate potentially active and total bacterial communities respectively. Moreover, next generation amplicon sequencing was used to compare potentially active and total communities in air to those in possible source and deposition environments. By contrasting source bacterial communities with atmospheric communities, we aimed at examining whether bacteria inhabiting certain environments, e.g. plant leaves or soils, are pre-adapted to activity under atmospheric conditions. Overall, our findings suggest that soils, decomposing vegetation, and plant surfaces feed the atmospheric boundary layer with bacterial cells that contain ribosomes, allowing them to actively interact with atmospheric processes in the Arctic region.

KEYWORDS: AIRBORNE BACTERIA, BIOGEOGRAPHY, DISPERSAL, BACTERIAL ACTIVITY, ATMOSPHERIC PROCESSES

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Poster B-36

BEDROCK AND BIOTIC INFLUENCE ON COMMUNITY COMPOSITION IN SOILS FROM THE SØR RONDANE MOUNTAINS, EAST ANTARCTICA

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Antarctica is a continent of extremes; the low availability of liquid water and nutrients, extreme low temperatures and seasonally variable levels of solar radiation exert high selective pressures on organisms. Consequently, most life forms in the scarce ice-free regions which represent less than 1% of the surface area of the continent are microbial. Despite this, terrestrial microbial communities are poorly studied and the existing data are geographically focused on McMurdo dry valleys and volcanically active regions. Nunataks, mountain tops protruding through the ice sheets, occur along much of the East Antarctic coast and in the Transantarctic Mountains. Among them, several remained ice-free during Neogene and Pleistocene ice ages and thus may have acted as important refugia for terrestrial life.

Here we present the results of a broad-scale survey of microbial biodiversity of ice-free regions in the western Sør Rondane Mountains (Dronning Maud Land (DML), East Antarctica). A total of 66 samples from eight different ice-free regions were selected to represent gradients in bedrock type (gneiss or granite), the macrobiotic content (presence or absence of moss, lichen and/or arthropods) and geographic location. All samples were subjected to both genetic fingerprinting (ARISA) and second generation sequencing (Illumina MiSeq 300PE) targeting the V1-V3 variable regions of the 16S rRNA gene. Mock communities were included to benchmark the bioinformatics pipeline. Reads were processed using Usearch (Edgar 2010), clustered based on a 97 % similarity cutoff using Uparse (Edgar 2013) and identified using the GreenGenes training set. The specific conductivity, pH, water content, and total (TC), total organic (TOC) and inorganic (IC) carbon content were determined and used as explanatory variables in direct ordination analyses of both the ARISA and the Illumina data.

The Illumina sequencing resulted in ~600.000 high quality sequences divided over ~3980 OTUs in 28 phyla and 219 genera. No significant differences in richness equaling the number of OTUs after standardization for the number of sequences per samples were observed between high, medium and low TOC content classes for the sequencing data. Redundancy Analysis revealed that bedrock type (granite or gneiss), water content, specific conductance, pH and TOC significantly shaped the bacterial community composition. The ARISA dataset, despite having a lower taxonomic resolution, showed very similar patterns and relationships with environmental data, among which bedrock type remained the most important parameter in explaining differences in community structure between the samples.

As the gneiss is supposedly of granite origin, differences in community structure may be related to physical differences between both bedrock types and their weathering products. Preliminary cosmogenic analysis of Pb isotopes of gravel samples indeed suggest a predominantly local origin of the material, yet mixtures with exotic material cannot be excluded in samples from gneiss outcrops. We conclude that microbial community composition is primarily driven by mineralogical characteristics of weathering products in these poorly developed soils, while biotic influences are of secondary importance.

KEYWORDS: TERRESTRIAL, EAST ANTARCTICA, SØR RONDANE MOUNTAINS, ILLUMINA, ARISA

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Poster B-37

BIOGEOGRAPHIC PATTERNS IN ANTARCTIC LACUSTRINE PROKARYOTES

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Amplified climate change, increased human activity and the introduction of alien species likely form the biggest threat to Antarctic terrestrial ecosystems through range size expansions and contractions, regional extirpation and impacts on ecosystem functions. Despite their crucial role in the functioning of Antarctic terrestrial ecosystems, little is known about the present-day diversity and biogeography of microorganisms such as prokaryotes and microeukaryotes in the Antarctic Biogeographic Realm. Furthermore, identification of the key processes underlying microbial biodiversity dynamics is essential to understand and predict the consequences of global change on Antarctic lacustrine ecosystems.

We analysed bacterial biodiversity in a total of 152 lacustrine microbial mat samples, distributed over the three main Biogeographic regions in the Antarctic Realm, including continental Antarctica, Maritime Antarctica and the Sub-Antarctic Islands comprising the southern Indian Ocean Province (SIOP) and the southern Pacific Ocean Province (SPOP). We targeted the V1-V3 variable regions of the 16S rRNA gene. Amplicon sequencing was done on an Illumina PE300 MiSeq. Sequences were processed using Usearch and Uparse, Mothur and custom scripts for basic parsing. An OTU cut-off was defined at 97 % sequence similarity, and sequences were mapped against a local GreenGenes database. Downstream analyses were performed using several R packages.

We obtained about three million high quality sequences, with an average length of 500 bp. Sequences belonged to 8237 OTUs, and were distributed over 51 phyla and 366 genera. In addition, 649 OTUs remained unclassified at the phylum level and 6263 at the genus level. Mean OTU richness differed strongly between the four biogeographic regions. The lakes from Maritime Antarctica had a higher richness than those from Continental Antarctica. Interestingly, in sub-Antarctica OTU richness was strongly variable, with Marion Island (SIOP) having the lowest and Macquarie Island (SPOP) having on average the highest diversity of all studied regions. Multivariate Analyses showed that microbial community composition varied between biogeographic regions, with Macquarie Island being most different from the other regions. Continental Antarctica, Maritime Antarctica and the lakes from the SIOP share many OTUs, both in the case of *Cyanobacteria* and other bacteria, but are also characterised by a considerable number of unique OTUs. Within Antarctica, some regions harbour distinct bacterial communities such as the lakes in Schirmacher Oasis, Dronning Maud Land, and those from the eastern and western part of the Antarctic Peninsula.

KEYWORDS: ANTARCTICA, BIOGEOGRAPHY, CONSERVATION REGIONS, PROKARYOTES, ILLUMINA

Poster B-38

A PLEA FOR THE CREATION OF INVIOLATE AREAS TO PROTECT REFERENCE AREAS FOR FUTURE MICROBIOLOGY RESEARCH IN ANTARCTICA

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Antarctica is essentially a microbial continent. A surprisingly large biodiversity of adapted microorganisms lives permanently in various biotopes of the ice-free areas (about 44,000 km^2). Based on molecular methods and microscopic observations, important findings like the presence of potentially endemic taxa, their survival in glacial refugia since the continent moved away from Australia and South America, and the determination of biogeographic patterns have been inferred. Moreover, Antarctic microorganisms may contain novel molecules with potentially pharmaceutical or biotechnological interest.

However, microbial habitats are under pressure as a result of anthropogenic introductions. Indeed, as a consequence of human presence, non-indigenous microorganisms are released from bodies, clothing, cargo and food into the environment (Cowan et al. 2011). The increase of tourism and its diversification from coastal cruises to adventurous expeditions into the continent, as well as the increase of research stations and associated impacts, constantly create new 'entry points' for microbial contamination (Chown et al. 2012). The impacts of such introductions are still unknown, and might lead to a loss of the native microbial biodiversity, or its modification by lateral gene transfer.

The technical progresses in molecular methodologies, like we currently see with Next Generation Sequencing (NGS), mean that very sensitive high-throughput analyses will become increasingly accessible. They have the potential to describe the microbial communities with unprecedented details without preconceived expectations. However, by that time, we might have lost the pristine Antarctic areas that would enable the scientists to study the native microbial flora, its functioning and properties.

The Protocol on Environmental Protection of the Antarctic Treaty foresees the designation of Antarctic Specially Protected Areas (ASPA) to protect "outstanding environmental, scientific, historic, aesthetic, or wilderness values, any combination of those values, or on-going or planned scientific research" (http://www.ats.aq/e/ep_protected.htm). However, the designation of ASPAs has not followed a systematic planning, and often focused on the conservation of large animals or higher plant communities. Microorganisms have the handicap of generally being invisible without a microscope and relevant expertise, and needing molecular methods to determine their identity. Terrestrial habitats are protected in 55 out of the 72 existing ASPAs (in total less than 700 km^2), mostly based on the need to protect vascular plants and bryophyte communities (Shaw et al. 2014). In 28 ASPAs, the protection targets the lichens, whereas microalgae are protected in 16 ASPAs, cyanobacteria in 7 and snow microalgae in 3. Only 8 ASPAs mention 'Microbial habitats', 'microbial communities' or 'soil and lake microflora'.

One tool of the Protocol that could be specifically used to protect microbial habitats is the creation of inviolate areas where no visitation is permitted (inside ASPAs, for example). These zones could be set aside for future research (Hughes et al. 2013) and become extremely valuable. After a few decades, they would be unique examples of truly pristine habitats, representative of the native microbial diversity and processes.

Such an option would necessitate discussions and consensus with scientists of other disciplines to select these regions, and careful management protocols of the sites and their vicinity (Hughes et al. 2015). In addition, gaps in knowledge should be addressed, like the extent of transportation of microorganisms by natural means (winds, birds...) (e.g. Pearce et al. 2009), and the probability of subsequent colonization of new areas by microorganisms coming from other Antarctic regions or from outside Antarctica. Let's hope that the dialogue between scientists and policy makers will enable to improve the conservation of Antarctic microbial diversity and safeguard the possibility to study these unique communities in the future with the most advanced techniques of the time. The outcome of these discussions might also be of interest for Arctic and alpine regions.

KEYWORDS: MICROBIAL DIVERSITY, CONSERVATION, INVIOLATE AREAS, ANTARCTICA, ASPA

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Poster B-39

BACTERIAL GEOGRAPHY OF HIGH ALTITUDE SNOW – COMPARING DIVERSITY IN SNOW FROM JUNGFRAUJOCH (SWITZERLAND) AND SNOWY MOUNTAINS (AUSTRALIA)

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Snow can be used to analyze the diversity of airborne organisms, as the classic dendritic form of the falling snow crystals efficiently scavenge any particles from the atmosphere (biotic and abiotic). A clear difference between aerially deposited bacterial assemblages at the snow surface and metabolically active bacteria in the snowpack has been shown in the Arctic. Here, we are interested in the comparison of bacterial diversity of high altitude mountain snow from the northern and southern hemisphere. Fresh surface snow samples (maximum 2 days old) from mountains in Switzerland (Jungfraujoch 3450 m.a.s.l. and Rosstock 1400 – 2360 m.a.s.l.) and the Australian Snowy Mountains (1500 – 2080 m.a.s.l.) were collected. All samples were carefully taken using sterile instruments as to avoid contamination. Snow samples were then left standing to melt, and filtered through membranes to collect the biomass. The latter was then subjected to DNA extraction and sequencing of the V1-V3 region of the 16S ribosomal RNA gene. Diversity was detected and analyzed using Qiime and the R software. Selected snow samples were also subjected to cell enumerations using fluorescence microscopy as well as enrichment and isolations of strains on diverse low nutrient media. Cell numbers averaged at 6.9×10^5 cells/ml ($\pm 7.5 \times 10^4$). Over 59 isolated strains of fungi or bacteria from 12 different samples were obtained. Paired-end sequencing of the 16S rRNA gene amplicon resulted in over 70‘000 sequences from 25 samples, ranging from 543 to 5173 sequences per sample.

Based on isolated strains as well as high throughput sequencing, we detect a great diversity of bacteria in samples from high altitude snow of the Swiss Alps as well as the Snow Mountains in Australia. The sequence data allows the comparison of the bacterial snow populations of a mountain from the northern and southern hemisphere and shows overlap and differences in community compositions. Also, the data provides insight into differences in bacterial populations across altitudinal gradients of the mountains. With the isolated strains, further studies on the survival mechanism and metabolisms of snow bacteria can be conducted. Knowledge on microbial diversity in snow from high altitude mountains is still very limited due to accessibility issues as well as only recent technological developments to analyze samples with extremely low biomass. To our knowledge, this is the first bacterial high-throughput sequencing dataset of snow from mountains of both hemispheres. Not only does this research provide an inventory of bacterial diversity in alpine snow but is provides answers to the geographic distribution and the origins of bacteria in snow.

KEYWORDS: SNOW, BACTERIAL DIVERSITY, BIOGEOGRAPHY, MOUNTAIN ECOLOGY, ALPINE MICROBIOLOGY

Poster B-40

HIGH OCCURRENCE OF THERMOPHILIC BACTERIA ISOLATED FROM HYDROTHERMAL VENT PLUMES IN AUSTRALIAN-ANTARCTIC RIDGE, SOUTHERN OCEAN

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INTRODUCTION
The intermediate spreading Australian-Antarctic Ridge (AAR) in the Southern Ocean is one of the largest unexplored regions of the global mid-ocean ridge systems because of limited accessibility due to harsh environment. Although activities of hydrothermal vents are supposed to be present in AAR, microbiological data are not available on cultivatable bacteria associated with those environments.
METHODS AND MATERIALS
During the IBRV Araon cruise in 2013, 14 samples of vent plumes were collected with Niskin bottles by CTD (conductivity, temperature and depth with potential reduction and turbidity sensors integrated) toyo-ing. Among the colonies grown on marine agar plates at 20°C from the vent samples, 139 colonies were randomly selected and purified for further investigations, determination of maximum temperature for growth and identification by 16S rRNA gene sequencing.
RESULTS
52 out of 139 (ca. 37%) strains were able to grow at 45°C, which are referred as thermophlic bacteria here, while the others were mesophiles. Among the isolated thermophlic bacteria, 42 strains were affiliated with *Firmicutes* and 9 strains with *Gamma-proteobacteria* based on 16S rRNA gene sequences. Mesophilic bacteria comprised of *Actinobacteria* (7 strains) and the above-mentioned groups (i.e. 17 and 64 strains for *Firmicutes* and *Gamma-proteobacteria*, respectively). Notably, two *Bacillus lichneformis* strains represented fast growing at the high temperature of 60°C, which are the first strains originated from deep-sea hydrothermal vent environments
DISCUSSION
As expected, thermophilic bacteria were abundant in the hydrothermal vent plumes. Gram-positive bacteria, *Firmicutes*, were dominant in the thermophilic bacteria in the study area, while *Proteobacteria* are often dominant in hydrothermal vents in other areas. This discrepancy might be attributed to different sources of vent fluids or different types of vent in respect to temperature and chemical compositions.

KEYWORDS: THERMOPHILE, BACTERIA, VENT, SOUTHERN OCEAN

Poster B-41

MOLECULAR CHARACTERIZATION OF MINIATURE PLASMIDS OF ARCTIC PSYCHROPHILIC BACTERIA OF THE GENUS *Variovorax*

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INTRODUCTION

The Svalbard archipelago is the northernmost landmass in the European Arctic and has a variety of small- and medium-sized glaciers. Among them are Hans and Werenskiold Glaciers, located on the south of Spitsbergen Island. Frequent and abundant inhabitants of this environment are bacteria of the family *Comamonadaceae* (*Betaproteobacteria*), including the genus *Variovorax* (Franzetti et al. 2013, Simon et a. 2009). We performed analysis of plasmidome of several psychrophilic strains of *Variovorax* spp., isolated from the ice glacier's surface of the Spitsbergen Island.

METHODS AND MATERIALS

Common microbiological and molecular methods were used to isolate and identify bacteria and isolate their plasmids. *In silico* methods (similarity searches, multiple sequence alignments, identification of conserved protein domains) were used for the analysis of the plasmid nucleotide sequences.

RESULTS

Numerous bacterial strains were isolated from the ice sample of the Hans and Werenskiold Glaciers. For more detailed analyses 14 strains, which formed yellow-coloured, smooth and circular colonies, were used. Based on the 16S rRNA gene sequence all the strains were assigned to the genus *Variovorax*. Further analyses revealed that these strains carry five plasmids, whose nucleotide sequences have been determined. Four plasmids, exhibiting high reciprocal sequence similarity, were miniature replicons of the size not exceeding 1 kb, including pVAR1 (746 bp) - the smallest autonomous replicon identified so far in free-living bacteria. The plasmids have no similarity with known sequences in the databases. *In silico* and experimental analyses allowed identification of DNA regions essential for the plasmids' replication.

DISCUSSION

Our study provided a valuable insight into the biology of bacterial plasmids. We showed that psychrophilic strains of *Variovorax* spp. carry related miniature plasmids of unique sequence and structure. The plasmids contain replication systems of a novel type. They do not encode any proteins, which strongly suggests that their replication might be initiated by regulatory RNA molecules.

KEYWORDS: PSYCHROPHILE, VARIOVORAX SP., MINIATURE PLASMID

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Poster B-42

DIVERSITY OF *pufM* AND *g5* GENES IN BACTERIOPLANKTON COMMUNITIES IN COASTAL SEAWATERS OF FILDES PENINSULA, KING GEORGE ISLAND, ANTARCTICA

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The bacterioplankon phylotypes of Alphaproteobacteria are among the largest heterotrophic marine bacteria and often detected in various marine regions on Earth. Order Rhodobacterales members have been found as most abundant members in temperate and cold waters (Buchan et al. 2005, Hagström et al. 2002). Known as aerobic anoxygenic phototrophic (AAP) bacteria, members of the Rhodobacterales (e.g. *Sulfitobacter* and *Loktanella*) possess bacteriochlorophyll *α* and are capable of utilizing dissolved organic substrates and harvesting light energy, though they are unable to grow autotrophically. Those bacteria can potentially serve important functions in marine carbon and energy cycling. As one of the genes coding for the subunits of reaction center complexe, *pufM* has been used as a gene marker to assess the diversity of different aerobic anoxygenic photosynthetic assemblages. Horizontal gene transfer is important in the evolution of bacterial genomes. Gene transfer agent (GTA)-related gene tranfer has been considered as a potential adaptive mechanism of these bacteria to maintain metabolic flexibility in changing marine environments. A capsid protein-encoding gene (*g5*) of GTA has been used as a marker to estimate the diversity of Rhodobacterales in temperate and cold waters because GTA genes are well conserved in Rhodobacterales. Although the distribution of the Rhodobacterales community in cold waters has been reported, information concerning the genetic diversity of Rhodobacterales in Antarctic waters remains insufficient.

In this study, pyrosequencing of the 16S ribosomal RNA gene (rDNA) amplicons was performed to investigate bacterioplankton community structure in Antartic Ardley Cove and Great Wall Cove during austral summer. Furthermore, the diversity of Rhodobacterales based on *pufM* and *g5* genes was investigated. Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria constituted the majority of bacteria. Representd by the genera *Sulfitobacter* and *Loktanella*, Rhodobacterales was the most dominant group (91%) in Alphaproteobacteria in the investigated area. Two *pufM* gene clone libraries were constructed from samples representing the two coves, and a total of 279 positive clones were obtained. Cloned sequences fell into Alphaproteobacteria, Betaproteobacteria classes and unidentified bacteria. Rhodobacteraceae-related *pufM* genes (93 % of the total *pufM* clones) were dominant in samples. In addition, the predominance of sequences (66% of the total *pufM* clones) closely related to *pufM* sequence encoded on a plasmid in *Sulfitobacter guttiformis* was observed in both samples. Two *g5* gene clone libraries were also constructed from samples representing the two coves and yielded diverse sequences. Sixteen *g5* clusters could be identified among 311 positive clones. *Sulfitobacter* and *Loktanella*-related *g5* sequences accounted for 52% and 33% of the total *g5* clones, respectively. Results in this study not only indicate an importance role of the Rhodobacterales as AAP bacteria in Antarctic coastal waters, but also suggest that the Rhodobacterales population could be highly diverse in that cold area. In addition, phylogenetic tree analysis of *pufM* and *g5* genes between Antartic and Arctic (Kongsfjorden) samples suggest a transpolar distribution of Rhodobacterales members in marine environments. At the same time, differences between Arctic and Antarctic sequences could support polar endemism.

KEYWORDS: *pufM* ; *g5*; BACTERIOPLANKTON COMMUNITY; ANTARCTICA

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Session C
Cold physiology and cryobiology

Keynote lecture KN-C

MICROBIAL ACTIVITY IN NEWLY THAWED PERMAFROST SOIL

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Permafrost covers 20% of the land area in the northern hemisphere and is estimated to contain 1700 billion tons of organic material equaling almost half of organic material in all soils. Due to global warming permafrost is thawing over large tracts of the Arctic, which may lead to increased emission of the greenhouse gases carbon dioxide, methane and nitrous oxide from soil to the atmosphere, potentially increasing global warming. We measured the release of carbon dioxide, methane and nitrous oxide from permafrost soil samples from NE Greenland and Svalbard incubated at 2 and -18 ^{o}C and related the release to changes in the abundance and community structure of potentially active bacteria. We found that the release of greenhouse gases from newly thawed permafrost was dominated by physical release of gases trapped in the frozen soil, while an increase in bacterial 16S rRNA copies indicated enhanced microbial activity one week after thawing. Based on our data, we propose a conceptual model for the release of greenhouse gases from newly thawed permafrost soil involving i) release of trapped gases, ii) enhanced microbial activity related to increase in liquid H_2O, and iii) long-term microbial degradation of organic matter.

Lecture C-01

CALLOSE ACTS AGAINST DESICCATION - INDUCED FORCES IN FILAMENTOUS STREOPTPHYTE ALGAE FROM ALPINE REGIONS

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Green algae colonised terrestrial habitats about 450 million years ago, giving rise to the evolution of land plants (Timme et al. 2012).[1] The filamentous streptophytes *Zygnema*, a member of the closest relatives to land plants (Zygnematophyceae), and *Klebsormidium*, belonging to the early branching Klebsormidiophyceae, occur worldwide in harsh aero-terrestrial habitats with unpredictable water supply (Holzinger et Karsten 2013). To date, the contribution of their cell walls to survive dehydration involving drastic forces to the cell and protoplast is poorly understood. We found that the β-D-1,3-glucan callose located in the cell walls of *Zygnema* and *Klebsormidium* (Sørensen et al. 2011) is essential to survive desiccation by making cell walls flexible to prevent mechanical damage. We used immunocytochemistry by means of confocal laser-scanning and transmission electron microscopy to visualize callose in *Zygnema* and *Klebsormidium* isolated from the Austrian Alps. Callose in both control and desiccated (30 or 210 min at ~65% relative humidity (RH)) algal filaments was quantified spectrofluorometrically. The effective quantum yield of PS II (Y(II); false colour image) and near infrared remission (780nm) in control, desiccated (30 min) and rehydrated (10, 60 and 180 min) individual filaments were visualized by a microscopic version of an Imaging-PAM. In *Zygnema*, callose was restricted to the longitudinal cell walls between individual cells and desiccation up to 210 min did not change the content, which was around 1.5 µg mg dry mass^{-1} in both strains investigated. The drastic deformation of the cells was reversible, indicated by recovering the Y(II) to ~50% of the initial value after rehydration. Filaments of *Klebsormidium* reduced their initial diameter after desiccation since the cross cell walls became undulated, while the outer walls were not deformed. Callose was mainly located in cross walls and desiccation increased the amounts from 3.0 to 6.3 µg mg dry mass^{-1} (*K. crenulatum*) and 4.9 to 8.3 µg mg dry mass^{-1} (*K. nitens*) already after 30 min. Rehydration of individual algal filaments allowed photosynthesis (Y(II)) to recover almost fully after 1 h (*K. nitens*) or 3 h (*K. crenulatum*), respectively.

The high abundance of callose in the cross walls of *Klebsormidium* allows a controlled reduction of the filament diameter during cellular water loss and the outer cell walls to remain closely attached to the protoplasts, which is crucial for preserving the structural integrity of the basic cell organelles[2] and a fast recovery of the Y(II) after rehydration. In *Zygnema*, callose-rich areas between individual cells likely act as supports and help dissipating shearing forces to avoid rupture of the load-bearing cell wall scaffold during desiccation. Our work demonstrates that specific components of cell walls in algae contribute to survive water loss, which played a key role in the transition from aquatic to terrestrial habitats.

ACKNOWLEDGEMENT: This work was supported by FWF grants P 24242-B16 and I 1951-B16 to A.H.

KEYWORDS: AERO-TERRESTRIAL GREEN ALGAE, CELL WALL, IMAGING-PAM, IMMUNOCYTOCHEMISTRY, TERRESTRIALIZATION

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Lecture C-02

META-OMIC ANALYSIS REVEALS WIDESPREAD FUNCTIONALITY IN ANTARCTIC HYPOLITHS FROM TWO DRY VALLEY SYSTEMS

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A substantial portion of Antarctica is covered by an expansive ice-sheet, with less than 4% of the continent being ice-free. These regions are mostly hyperarid and potentially store a vast amount of terrestrial carbon. The microbial communities in these soils, and associated cryptic niche habitats, are relatively underexplored and it remains completely unknown how they are likely to respond to changing climatic regimes. The studies, which have been undertaken in these terrestrial systems have been primarily DNA based, which could represent historical signatures of DNA. To decipher the relationship between community structure and function, we applied metagenomics, metaproteomics and metabolomics to hypoliths and soils from two Antarctic Dry Valleys. Targeted analysis of 16S rRNA gene amplicons from hypoliths and soils indicated broadly similar microbial phylogenetic composition. However, results from both metaproteomics and metabolomics clearly indicated highly distinct functional microbial communities and biogeochemical cycling processes, which were largely driven by hypolithic communities (niche effect), with very little activity in soils. To elucidate the physiological capacity of hypolithic systems we reconstructed thirteen different genomes belonging to *Actinobacteria*, *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Planctomycetes*, *Gemmatimonadetes* and *Verrucomicrobia*. Analysis of these genomes indicated that five of these harbored a photosystem. Interestingly, these genomes included three with a rhodopsin-like system, which has never been reported in edaphic systems. These results provide the first evidence of microbial functionality (c.f. to functional capacity) in depauperate Antarctic systems and allow us to better predict how soil communities may respond to changing climates.

Lecture C-03

SOME LIKE IT COLD, SOME LIKE IT GREEN, SOME LIKE IT COLD AND GREEN - COMPARATIVE GENOMICS OF SPHINGOMONADS ASSOCIATED WITH ARCTIC PLANTS

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Bacteria in the genus *Sphingomonas* are known to be metabolically versatile and successfully occupy diverse ecological niches. They are typical in oligotrophic environments, but have also been reported to dominate plant phyllosphere communities (Kim et al. 1998, Delmotte et al. 2009). *Sphingomonas* spp. are also abundant in the endophyte communities of arcto-alpine pioneer plant species *Oxyria digyna* and *Diapensia lapponica* (Nissinen et al. 2012). These *Spingomonas* spp. are pshychrotolerant, host plant specific, and are tightly associated with their respective host plants. We have isolated them repeatedly from the phyllosphere, the endosphere and seed tissues of their respective host plants in different years, in many sampling sites located in Kilpisjärvi, Finland and in Longyearbyen, Svalbard. Based on their 16S rRNA sequence, the *Sphingomonas* sp. strains associated with *D. lapponica* are closely related to *S. glacialis*, in particular to strains isolated from arctic lichens, alpine glaciers and antarctic soils. The closest relatives of *O. digyna* –associated *Sphingomonas* sp. are related to *S. aurantiaca* and *S. faeni*, and have been isolated from arctic rhizosphere soils, Antarctica and alpine glaciers.
In order to better understand their adaptation to host plants and their putative role in plant adaptation to arctic habitats, we acquired high quality draft level genome sequences of three representative strains of both *Sphingomonas* species. We used genome mining and comparative genomics with other publicly available *Sphingomonas* spp. genomes from plants, lichens and soils, as well as from temperate and cold climates in order to detect plant (host) and climate specific features.
Pathways dedicated to production or manipulation of plant hormones, as well as plant defense compounds were prominent in the arctic endophytic *Sphingomonas* genomes. A wide repertoire of genes dedicated to abiotic stressors, including oxidative stress, heat and cold shock and metal detoxification were typical for all the arctic strains. We also detected host plant specific features, for example detoxification of specific plant phenolic compounds, plant hormone production, nitrogen fixation and potential for anoxygenic photosynthesis. Production of bacteriochlorophyll A was detected in several *D. lapponica*-associated phyllospheric and endophytic strains.

KEYWORDS: ENDOPHYTIC BACTERIA, SPHINGOMONAS, ARCTIC, GENOME

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Lecture C-04

NITROGENASE ACTIVITY OF SOIL CYANOBACTERIAL CRUSTS IN POLAR AND SUBPOLAR URALS (EUROPEAN NORTH-EAST RUSSIA)

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Biological soil crusts, formed by cyanobacteria, algae, fungi, lichens and mosses, have a significant part in formation of vegetation cover in high-latitude regions. Due to unique ability to fix carbon dioxide and nitrogen, cyanobacteria are important functional component in soil crust communities. It is essential to assess contribution of cyanobacterial crusts into carbon and nitrogen balance since data are necessary for simulations of global processes and climate change prediction, occurring in high mountain regions. During research, new data was obtained on cyanobacteria crusts and their role in different types of mountain tundra in European part of the Russian Arctic. A field study was conducted to investigate nitrogenase activity (NA) during the day. In 2013-2014, daily NA measurements were performed in biological soil crusts with dominance of cyanobacteria. The samples were collected in mountain tundra on the northern tip of the Polar Urals (68°28' N, 66°22' E, 165 m above sea level), and in the mountain-tundra belt of Subpolar Urals (65°11' N, 60°18' E, 680 - 1305 m). The measurements were performed in the third week of July for two days: in Polar Urals during sunny and unseasonably warm days with an average temperature of 20 ^{0}C, in Subpolar Urals in the typical for the area days with daily average temperature of 10 ^{0}C. In both areas, two different types of soil crusts were chosen: V1 – crusts from low moisture areas, with dominance of *Stigonema* (*S. ocellatum* in the Polar Urals, *S. minutum* and *S. ocellatum* in the Subpolar Urals), V2 – crusts from wet habitats where water supply permanently comes from glacier melt waters or wetlands, species from *Nostoc* and *Scytonema* genera (*N. commune* and *Sc. ocellatum*) dominated. Pieces of 3x4 cm^2 (with mineral soil layer thickness of 1 cm) were cut from cyanobacterial crusts. For each variant the sampling was replicated 3 times. Afterwards, samples were placed in 130 ml conical flasks, the temperature inside them was measured using autonomous recorder DS-1921. The flasks were sealed, 10 ml of air was extracted and afterwards 100 ml of 13% of acetylene were added to the flask to create 10% of acetylene mixture. The first sample of gas mixture was taken after 10 minutes after addition of acetylene, the second sample – after 2 hours; each time 3 ml sample was taken and injected into 6 ml Labco Exetainer flasks. Temperature of the crusts in flasks was approximately the same with environment temperature at the time of experiment. Measurements were carried out for two days during given times: from 2:30 to 4:30, 7:30 to 9:30, 13: 30 to 15:30, 18:30 to 20:30. After the measurement, the sample was placed back to the soil until the next measurement. Parameters of surrounding microclimate (temperature, relative humidity and active radiation) were recorded by automatic micrometeorological station HOBO. The analysis of ethylene was performed in a flask in laboratory, using gas chromatograph Zvet-800 (Russia) with Porapak N 80/100 sorbent in metal 2 m column. Standard Linde Gas mixtures were used to calibrate Zvet-800. Statistical analysis was performed by Statistica 6.0 program.

During day, NA was optimal in early evening, when the temperature was at its maximum (Fig. 1A). In the evening, just after the sunset, NA was reduced by 50-55% of the daily values. Additional studies (where temperature was constant) showed that the decline in this period was mainly caused by changes in the light regime after the sunset. During the night, NA was on sufficiently high level, despite the absence of light for 6 hours, and at the time of sunrise, NA was 38-41% of the daily values. In the morning, NA recovered quickly, by 8:00 am it was reaching 80% of the maximum daily values, restricted by temperature conditions. Under constant humidity, daily dynamics of NA in the crust correlates with the temperature at the time of the measurement, daily NA correlates with average daily temperature of the day ($R=0.80$, $p<0.05$, $n=15$), the maximum values of NA during the day with average daily temperatures of the previous day ($R=0.85$, $p<0.05$, $n=29$). It was noted V2 crusts from both locations had significantly higher rates of NA, comparing to V1 crusts. On the other hand, there was nospecific difference within the V1 or V2 groups in NA rate. The table shows the comparative results, obtained from measurements under 13 ^{0}C and 20 ^{0}C. The daily NA was calculated by approximating curves of the NA daily dynamics by polynomial function (Fig. 1A). NA for the night period (from 21 pm to 3 am when the measurements were not carried out) was calculated from the correlation between temperature and NA (Fig. 1B). Daily average values of NA in crusts for Polar and Subpolar Urals for V1 were 47 and 24.4 mg C_2H_4 m^{-2}d^{-1}, respectively, and for V2 - 66.9 and 55.9 mg C_2H_4 m^{-2}d^{-1}. The relatively high rate of daily NA in sample from Polar Urals was caused by higher temperature conditions during the period of measurement (table).

To compare, in Bolschezemelskaja tundra (67°35 'N, 63°47' E) NA speed in cyanobacterial crusts under 13 °C was 1.06 mg C_2H_4 m^{-2} h^{-1} (Patova et Sivkov, 2002), the values were close to the data, obtained from Ural samples (0.97-1.79 mg m^{-2} h^{-1}) under the same temperature (Table). In high Arctic (Ny-Ålesund, Svalbard, 78.5 °N, 11.6 °E) in

studies done T. Liengen (1999), daily NA ranged from 12.8-63.7 mg C_2H_4 m^{-2} d^{-1} under 19 ° C and 200 mkE m^{-2} s^{-1}. The data in our study (24.4 - 66.9 mg C_2H_4 m^{-2} d^{-1}) fall in the same range of values, obtained by Liengen.
Thus, for the first time, experimental data on daily nitrogen-fixing activity was obtained for cyanobacterial crusts in typical soil crusts of Subpolar and Polar Ural. Crusts with dominance of *Nostoc* and *Scytonema* showed a significant higher NA in comparison with crust with dominance of *Stigonema* genus. Perhaps, the difference could be explained by variety in projective cover of nitrogen-fixing cyanobacteria. There were no significant difference in daily NA values nor in specific speeds of NA in crust from Urals, comparing with results for the other regions of the Arctic and southern tundra. Our results can be used for calculations of seasonal activity of cyanobacterial crusts Ural mountain tundra and close the gap in research on nitrogen metabolism of this area.

ACKNOWLEDGEMENT: The research was supported by a grant Russian Foundation for Basic Research № 15-04-06346.

KEYWORDS: NITROGENASE ACTIVITY OF CYANOBACTERIA, POLAR AND SUBPOLAR URAL

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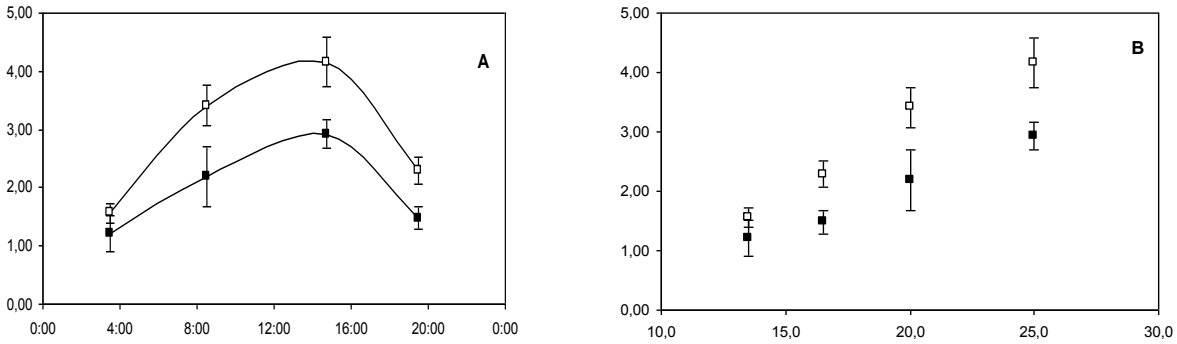

Fig. 1. A typical daily dynamics of nitrogenase activity (A) and correlation with temperature (B) nitrogenase activity in the Polar Urals cyanobacteria crusts example: ■ - for V1 (*Stigonema*), □ - for V2 (*Nostoc* and *Scytonema*). X-axis: time (**A**), the temperature ^{0}C (B), vertical axis - nitrogenase activity, mg C_2H_4 $m^{-2}h^{-1}$. Mean ± SD, n = 4

Table. Nitrogenase activity in cyanobacteria crusts under 20 °C (NA_{20}) and 13 °C (NA_{13}), mg C_2H_4 $m^{-2}h^{-1}$, the daily activity of nitrogenase NA_d, mg C_2H_4 $m^{-2}d^{-1}$, the average daily value of photosynthetically active radiation (µmol $m^{-2}s^{-1}$) and the temperature T_d, °C, mean ± SD, n = 3.

Index	Polar Ural		Subpolar Ural	
	V1	V2	V1	V2
	Stigonema	*Scytonema,Nostoc*	*Stigonema*	*Scytonema, Nostoc*
NA_{20}	2.19±0.52	3.41±0.26	2.68±0.17	3.83±0.54
NA_{13}	1.21±0.31	1.56±0.28	0.97±0.36	1.79±0.34
NA_d	47±7.5	66.9±10.7	24.4±4.8	55.9±6.3
FAR_d	390		460	
T_d	19.6		10.8	

Lecture C-05

THE ICE-BINDING PROTEINS OF A SNOW ALGA, *Chloromonas brevispina*: PROBABLE ACQUISITION BY HORIZONTAL GENE TRANSFER

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All ice-and snow-related unicellular algae examined so far secrete ice-binding proteins (IBPs) to mitigate freezing damage. Two types of IBP have been identified in chlorophytes. Type 1 IBPs are members of a large family of proteins that share a large domain of unknown function (DUF3494). Previous studies have suggested that the type 1 algal IBP genes were acquired by horizontal gene transfer. To test this hypothesis I sequenced the IBP genes of a snow alga, *Chloromonas brevispina*. that has strong IBP activity (Fig. 1a). The IBPs were identified by ice affinity purification, de novo sequencing of a tryptic peptide and large-scale sequencing of the transcriptome and genome. *C. brevispina* has genes for over 20 IBP isoforms, which strongly indicates their importance. The IBPs are all of type 1 and match fungal and bacterial proteins more closely than they match known algal IBPs, providing further evidence that the genes were acquired by horizontal transfer. Modeling of the 3D structures of the IBPs based on the known structure of a homologous protein suggests that the ice-binding site of C. brevispina IBPs (Fig. 1b) has characteristics that are shared by all DUF3494 proteins.

KEYWORDS: ICE-BINDING PROTEINS, *Chloromonas brevispina*, SNOW ALGA, DUF3494

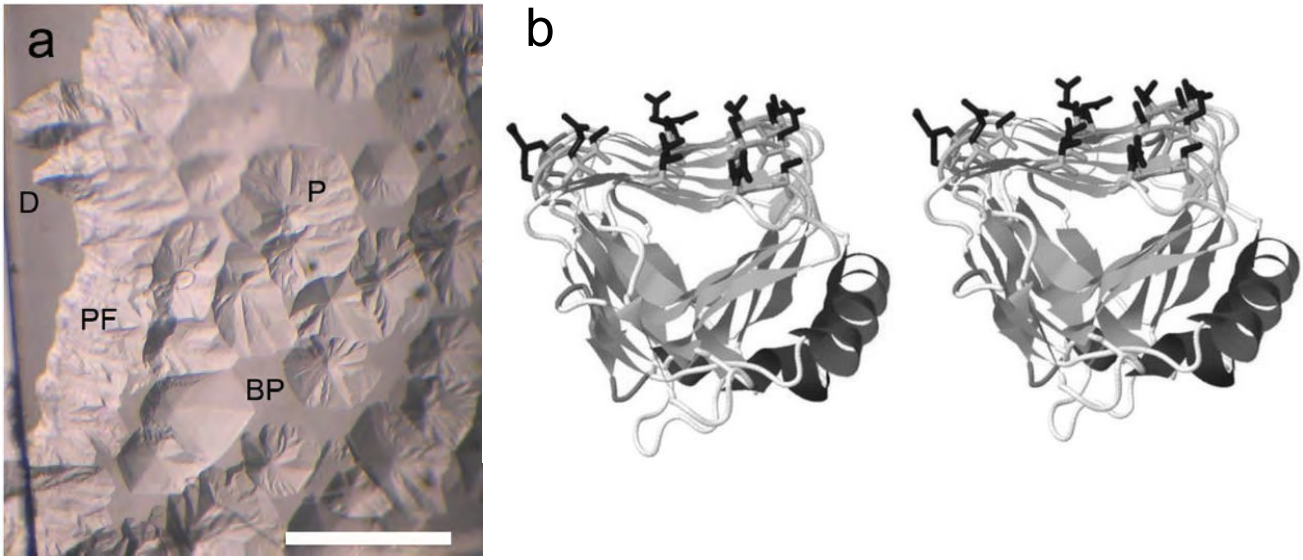

Fig. 1. Ice-binding proteins of *Chloromonas brevispina* . **a**, Effects of IBPs on the growth of an ice single crystal. The ice basal plane (BP) is parallel to the plane of the image. Characteristic ice-binding features include pits (P) on the basal plane and greatly distorted dendrites (D) growing on the prism faces (PF) of ice. Scale bar, 1 mm. **b,** Stereoview of predicted structure of a *C. brevispina* DUF3494-type IBP. It has the shape of a triangular prism with one of the three faces (top) having rows of hydrophilic amino acid residues that bind to ice.

Lecture C-06

THE LIMITS OF DESICCATION TOLERANCE OF ARCTIC *Microcoleus* STRAINS (CYANOBACTERIA) AND ENVIRONMENTAL FACTORS INDUCING IT

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Microcoleus species are among the most common cyanobacteria in water-deficient habitats, including hot and cold deserts. Surprisingly, there is nearly no information about degree of dehydration, which *Microcoleus* species are able to tolerate, survival rate of cells, damage that cells sustain upon desiccation, and environmental factors inducing their resistance to drying.

We tested three *Microcoleus* species isolated from terrestrial habitats of the High Arctic, for (i) their ability to survive complete and incomplete desiccation; (ii) investigated whether desiccation tolerance is their constitutive trait or it is induced under suboptimal conditions; (iii) determined the proportion of live cells, and their physiological state before desiccation and after rehydration. We cultivated the cultures in thin biofilms to provide them homogeneous conditions for growth and subsequent desiccation. Detection of desiccation survivors and investigation of their cellular function were carried out by direct cell counts in combination with multicolor fluorescence staining.

Three studied strains showed strikingly similar pattern of their response to drying:

(i) *Microcoleus* species did not survive complete desiccation (<0.1 g water g^{-1} dry biomass) regardless of cultivation condition we employed.

(ii) Nevertheless, the strains tolerated extensive dehydration (to only 0.23 g water g^{-1} dry biomass), which removes >90% of initial cell water.

(iii) Survival of incomplete desiccation was strongly dependent on cultivation conditions prior to desiccation, i.e. the strains do not possess constitutive desiccation tolerance. The optimally grown cultures showed 100% mortality, low temperature enabled only 0 to 15% of cells to survive, while after nitrogen starvation up to 65% of cells remained viable.

(iv) The cells that survived desiccation, maintained membrane integrity, resumed respiration short time after rehydration, did not differ morphologically from non-viable cells, and were subsequently able to grow under standard cultivation conditions.

We consider that unlike *Nostoc* and *Chroococcidiopsis*, which co-exist with *Microcoleus* in Arctic terrestrial habitats, *Microcoleus* species are not truly anhydrobiotic and do not possess constitutive desiccation tolerance. Instead, it seems that the survival strategy of *Microcoleus* in periodically dry habitats involves avoidance of complete desiccation, but tolerance to milder desiccation stress, which is induced by suboptimal conditions (e.g., nitrogen starvation). Avoidance of complete desiccation is realized through their community structure, migration of trichomes, and the presence of water-retaining sheaths. These results are in accordance with our field observations, which showed that a substantial proportion of cells in natural populations in the High Arctic readily survived freeze-induced dehydration and seasonal desiccation. The viable cells did not alter their morphology and ultrastructure, and quickly resumed respiration after melting or rehydration.

Lecture C-07

COPING WITH COLD: FROM THE STRUCTURE TO THE FUNCTION OF ANTARCTIC BACTERIAL GLOBINS

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INTRODUCTION

Since only about half of the 2200 bacterial genomes sequenced so far include genes encoding globins, it appears that these proteins may not always be required to sustain bacterial physiology; on the other hand, many bacterial genomes comprise more than one globin gene (Vinogradov et al. 2013). Truncated haemoglobins build one of the three branches of the globin protein superfamily. They display a characteristic two-on-two α-helical sandwich fold and are clustered into three groups (I, II, and III). *Pseudoalteromonas haloplanktis* TAC125 (*Ph*TAC125), a cold-adapted Antarctic marine bacterium hosts one flavohaemoglobin and three distinct truncated haemoglobins. To our knowledge, *Ph*TAC125 is the first example of coexistence of multiple globin genes (Giordano et al. 2013).The high number of globins suggests that these proteins fulfil crucial functional roles, perhaps related to the peculiar features of an O_2-rich and cold habitat as the Antarctic Ocean. In fact, gene redundancy has been shown to entail mechanisms that warrant expression of an essential function; such redundancy may be perceived as a systemic adaptation to extreme environments.

METHODS AND MATERIAL

One of these globins, encoded by the *PSHAa0030* gene (*Ph*-2/2HbO), has been extensively studied by spectroscopic studies, kinetic measurements, computer simulation approaches (Giordano et al. 2013) and very recently by X-ray diffraction.

RESULTS & DISCUSSION

Altogether, the results show that *Ph*-2/2HbO maintains the general structural features of group-II truncated haemoglobins, but displays enhanced conformational flexibility related to the O_2 concentration and low kinetic energy of molecules, experienced by organisms living in the Antarctic environment (Giordano et al., under revision).

Recent results on a genomic mutant strain highlight that the inactivation of the gene encoding *Ph*-2/2HbO made *Ph*TAC125 sensitive to high O_2 levels, hydrogen peroxide, and nitrosylating agents. In order to investigate the physiological role of these Antarctic bacterial globins and highlight their involvement in the NO detoxification mechanisms, the three globin genes have been cloned and over-expressed in the mutant of *Escherichia coli* defective in the flavohaemoglobin gene (*E. coli hmp*), hypersensitive to nitrosative stress. The NO-sensitive *E. coli hmp* cells expressing the cold-adapted globin genes were characterised by analysing the ability of the bacteria to grow and respire in presence of NO.

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Poster C-01

AN ANTARCTIC BACTERIUM WITH AN ICE ADHESION

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Ice-Binding Proteins (IBPs) are usually small, soluble proteins expressed in cold-adapted organisms to protect them from freeze threats by several biological strategies. In fish and freeze-avoiding insects, IBPs are known as antifreeze proteins because they depress the animal's freezing temperature.. In freeze-tolerant plants and in microorganisms, IBPs protect the cytoplasm from lethal growth of large ice crystals by inhibition of ice recrystallization. Microorganisms living in Antarctic sea ice secrete IBPs that are thought to have a role in keeping the immediate microenvironments somewhat fluid.

The IBP of the Antarctic bacterium *Marinomonas Primoryensis* is a tremendously large, 1.5-MDa protein consisting of five different regions. Most of the protein is a repetitive region that extends its C-terminal end away from the bacterial surface. Near the C terminus is a 34-KDa domain, which is responsible for ice binding. When recombinantly expressed as an independent unit, the IBP domain functions like an insect antifreeze protein. However, structural and bioinformatic studies suggest that this IBP serves as an adhesin that binds the bacteria to ice. We suggest that this adhesin helps the bacteria to gather at the upper layer of the water column, where oxygen and nutrients are available. This is a remarkable adaptation of the adhesin principle used by bacteria to bind to many kinds of surfaces. This is currently the only example where an adhesin has evolved to bind ice.

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Poster C-02

RESISTANCE OF ANTARCTIC *Nostoc* sp. COLONIES TO DEHYDRATION ASSESSED BY CHLOROPHYLL FLUORESCENCE PARAMETERS AND SPECTRAL REFLECTANCE

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INTRODUCTION

It is well established that spectral reflectance and photosynthetic parameters are dependent on actual hydration of a thallus, its relative water content (RWC) in autotrophic poikilohydric organisms. In *Nostoc* sp. from polar regions, these parameters have been investigated (see *e.g.* Moudrá et Barták 2009, Kvíderová et al. 2011, Trnková et al. 2014, Trnková et Barták 2015). In this study, we focused on a detailed analysis of NDVI and PRI spectral indices, and effective quantum yield of photosynthetic processes in photosystem II in desiccating *Nostoc* sp.

MATERIAL AND METHODS

Nostoc sp. colonies were collected from a seepage at the Long-term research plot (LTRP) located close to J.G. Mendel station (James Ross Island, Antarctics) in February 2015. Resistance to dehydration was assessed by the below-specified measurements on gradually desiccating samples from fully wet to fully dry state. During desiccation, hydration state was expressed as RWC using fresh weight measurements. Photochemical reflectance index (PRI) and Normanized difference vegetation index (NDVI) were measured by a PlantPen PRI/NDVI 200 (Photon System Instruments, Czech Republic) using the below equations and reflectance in particular wavelenghts.

NDVI = (R740–R660)/(R740+R660)
PRI = (R570–R531)/(R570+R531)

Effective quantum yield of PS II was measured repeatedly during dehydration period in 5 min. interval to evaluate optimum hydration for photosynthetic processes and critical point at extremely low water contents in *Nostoc* colony. For the measurements, a PAM-2000 ((H.Walz, Germany) fluorometer was used as described in Barták et al. (2015).

RESULTS AND DISCUSSION

NDVI showed a curvilinear relationship to RWC having a maximum of 0.4 at RWC of 55%. Towards low water content in the *Nostoc* colony (RWC of about 10 %), NDVI decreased to a minimum of 0.25. PRI increased slightly with colony dehydration. PRI minimum of -0.025 was found in 100% RWC, and reached maximum of 0.020 at dry state (RWC= 0%). At full hydration, effective quantum yield of *Nostoc* colony showed reduced values (to about 30% of maximum found at RWC range of 30%-50%). With further dehydration (typically below 20% of RWC), effective quantum yield showed a dramatic decrease to 0, *i.e.*full inhibition of photosynthetic processes. In conclusion, *Nostoc* sp. exhibited a high degree of resistence to dehydration.

KEYWORDS: DESICCATION, PRI, NDVI, *NOSTOC*, YIELD OF PHOTOSYSTEM II

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Poster C-03

DO *Chlorella* STRAINS RESPOND DIFFERENTLY TO TEMPERATURE STRESS ACROSS A GLOBAL GRADIENT?

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INTRODUCTION

Rapid trends of environmental change are especially apparent in parts of the polar regions, where temperature change as well as the melting of ice can alter ecosystems, thus affecting the algae that form the basis of aquatic food webs. Although previous work (Teoh et al. 2013) has demonstrated that *Chlorella* strains obtained from Antarctic, temperate and tropical regions are eurythermal and have considerable overlap in their temperature tolerances, the unique adaptive mechanisms that these algae employ to thrive under different temperature regimes and their potential capacity to acclimate to environmental change remain unexplored. The objective of this study was to investigate and compare the physiological and photosynthetic responses of similar *Chlorella* strains from Antarctic (*Chlorella* UMACC 237), Arctic (*Chlorella* UMACC 263), temperate (*Chlorella vulgaris* UMACC 248) and tropical (*Chlorella vulgaris* UMACC 001) locations to temperature stress as might be experienced under future warming scenarios.

MATERIALS AND METHODS

Algae in culture were exposed to four different temperature treatments: ambient (control, appropriate for the source of each strain), ambient + 4°C, ambient + 6°C and ambient + 8°C. The ambient temperatures used for each region were 4°C (polar regions), 18°C (temperate) and 28°C (tropical). The growth characteristics and photosynthetic performance of each strain were determined using spectrophotometry and PAM fluorometry, respectively.

RESULTS

The data obtained demonstrated that all the strains were able to adapt and survive under the experimental warming scenarios. Temperature elevation of 4°C did not affect the specific growth rate (μ) of either polar strain, while it led to higher (7.1–8.3%) μ of both temperate and tropical strains. Further elevation led to a decrease in μ of all except the Antarctic strain. However, maximum quantum yield (F_v/F_m) values indicated that all the strains remained in healthy condition with no indication of stress. The Antarctic and Arctic *Chlorella* strains achieved the highest photosynthesic efficiency (α) under ambient conditions. Elevation of up to 8°C in culture temperature led to 9.5–13.8% decrease in efficiency from the maximum levels observed, indicating considerable resilience to temperature variation in their natural aquatic habitats, even in tropical and polar regions where typical algal habitats can be thermally very stable. However, the reverse was observed in the temperate strain, with 25.2% lower efficiency observed under ambient conditions and highest levels in culture under 8°C of temperature elevation. In the tropical *Chlorella*, the greatest NPQ (non-photochemical quenching) value was observed at the highest experimental temperature.

DISCUSSION

Chlorella strains obtained from regions spanning a considerable range of environmental variation showed very similar levels of photosynthetic performance at their respective ambient temperatures, indicating the importance of thermal adaptation and compensation over evolutionary timescales in photosynthetic biochemistry. All the strains examined shared important features in their responses to experimental thermal elevation, in particular being able to survive considerable (8°C) elevation and in showing limited (Arctic, temperate, tropical) or no (Antarctic) change in growth rate. The relatively small, though significant, proportional changes in photosynthetic performance also indicate relatively flat responses to temperature variation over quite a wide range, suggesting these algae share considerable resilience to temperature change.

KEYWORDS: TEMPERATURE, *Chlorella*, POLAR, TEMPERATE, TROPICAL

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Teoh, M.L., Phang, S.M., Chu, W.L. (2013): Response of Antarctic, temperate and tropical microalgae to temperature stress. Journal of Applied Phycology 25 (1): 285-297

Poster C-04

ISOLATION AND SOME UNIQUE PHYSIOLOGICAL CHARACTERISTICS OF PSYCHROTROPIC FUNGI FROM PASSU GLACIER, PAKISTAN

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The Passu glacier has not been investigated earlier for fungal diversity. The current study involves the isolation and identification of psychrotrophic fungi from glacial ice, water and sediment samples collected from Passu glacier, Pakistan, and study of some important characteristics related to their physiological requirements and growth. Conventional and molecular techniques (18S rDNA sequencing), were used to identify fungi. A total of 25 fungal strains were identified. The most predominant genus was *Penicillium* (8), followed by *Mrakia* (3), *Cladosporium* (2), *Pseudeurotium* (2), *Fontanospora* (2), *Trichoderma* (1), *Antrodia* (1), *Sporobolomyces* (1), *Phoma* (1), *Beauveria* (1) and *Geomyces* (1) while one isolate belonged *Dothideomycetes* class. Tolerance of all strains to wide pH, temperature and salt concentration ranges was studied. All the fungal strains showed growth between 4 and 37°C, whereas some fungal strains were able to grow at 45°C. Most of the strains (~80%) showed growth at pH 1- 13 except 5 isolates that could not tolerate pH 1. Fungal isolates tolerated salt concentration between 2-26% with maximum range of all as 16% and above, i.e. all moderate to extreme halophiles. Fungal isolates were screened for their antimicrobial activity against clinically isolated bacterial and fungal strains and the findings were quite promising. *Fontanospora* sp. was able to show activity against *Staphylococcus aureus* and *Candida* sp. Fungal strains were screened for the production of extracellular enzymes (amylase, cellulase, deoxyribonuclease, lipase, phosphatase and protease) of valuable commercial and economic importance. Many strains were able to produce one or more enzyme, whereas, *Sporobolomyces ruberrimus* produced all enzymes except lipase. The study concludes that previously unexplored Passu glacier is a home for diverse types of fungi and the reported fungal strains are psychrotolerant, acidophilic/alkaliphilic, salt tolerant and have a great potential to produce novel antimicrobial metabolites and extracellular enzymes.

KEYWORDS: PASSU GLACIER, PSYCHROTROPHIC FUNGI, FUNGAL DIVERSITY

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Poster C-05

BIOTIC AND ABIOTIC CONTROLS ON THE ELEMENTAL AND ISOTOPIC COMPOSITION OF MICROBIAL COMMUNITIES IN MCMURDO DRY VALLEY STREAMS, ANTARCTICA

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INTRODUCTION

Quantifying isotopic and elemental composition of primary producers is a useful approach to investigating ecosystem foodwebs and nutrient mass balance. However, taxonomic differences, upper trophic levels, and habitat heterogeneity complicate mixing models, and disentangling these variables in field studies is difficult. Here we test biotic and abiotic controls on nutrient cycles by investigating the role of taxonomic identity, spatial position, and dissolved carbon (C), nitrogen (N) and phosphorus (P) concentrations on microbial mat elemental composition and isotopic ratios in the simple stream systems of the McMurdo Dry Valleys, Antarctica.

METHODS AND MATERIALS

Dry Valley streams, which have no upper trophic levels, riparian vegetation, or allochthonous inputs, vary in their nutrient concentrations in two predictable ways: First, N and P decrease from upstream to downstream due to microbial uptake, and this pattern is more pronounced for longer streams and those with higher standing microbial biomass. Secondly, dissolved N increases inland over Taylor Valley as a result of greater accumulated atmospheric deposition of N on older surfaces, while P is elevated towards the coast because of greater apatite deposits. We sampled three different microbial community types over these gradients; chlorophytes, *Nostoc*, and *Oscillatorean* mats. These were analyzed for their C:N:P content, and $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures.

RESULTS

Nostoc consistently exhibited the highest C:N, C:P, and N:P ratios, while *Oscillatorean* mats contained greater P (lower in C:P and N:P ratios). Chlorophytes had the most depleted $\delta^{13}C$ signatures and *Oscillatorean* mats the most enriched, which may suggest greater utilization of CO_2 in the former and HCO_3^- use in the latter. *Nostoc* was intermediate in $\delta^{13}C$ ratios, but more near the $\delta^{15}N$ atmospheric standard compared to other communities indicating N-fixation. Samples taken over a longitudinal stream gradient showed different patterns in elemental composition by community type. *Nostoc* nutrient ratios (C:N:P) were greater upstream than downstream, while patterns in *Oscillatorean* mats were opposite. The $\delta^{15}N$ signatures of *Nostoc* remained near the atmospheric standard (~0) over the length of the stream, indicating N-fixation. In contrast, *Oscillatorean* mats were much more depleted upstream, and aligned with the more enriched values for *Nostoc* at downstream reaches. Over the Taylor Valley gradient, elemental and isotopic ratios were sometimes strongly correlated to water column nutrient concentrations, but these relationships differed by community type. Molar C:P and N:P ratios decreased with greater P availability, but this was most pronounced for chlorophytes and *Oscillatorean* mats. *Nostoc* elemental composition was most correlated with N availability. The $\delta^{15}N$ signatures of all community types became more depleted as bulk N concentration increased as Taylor Valley extends inland.

DISCUSSION

Collectively, these data suggest that microbial mats assimilate nutrients as a function of the life histories of the dominant taxa, creating differential nutrient requirements and resource access. Furthermore, biotic and abiotic processes (such as denitrification and atmospheric deposition, respectively) have a substantial influence on the quantity and type of nutrients that are available to consumers. N is tightly cycled in N-limited streams, and N-fixing microbes may be responsible for substantial new N entering the system. These results aid in our understanding of stream biogeochemical cycles and ecosystem connectivity in polar regions, as well as lotic systems globally.

KEYWORDS: CYANOBACTERIA, ALGAE, BIOGEOCHEMISTRY, NITROGEN, ECOLOGY

Poster C-06

CHARATERIZATION OF PLASMIDS AND PLASMID-ENCODED RESISTANCE GENES FOUND IN PERMAFROST *Acinetobacter lwoffii* STRAINS

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INTRODUCTION

Bacterial plasmids are one of the key players in the process of horizontal gene transfer. They often carry accessory genes determining various adaptive phenotypes, such as resistance to toxic heavy metal compounds and antibiotics. These genes are often part of transposable and integrative mobile elements forming complex genetic structures that promote bacterial diversification and adaptation. To understand the evolutionary processes caused by the human impact on environment a molecular analysis of plasmids and genetic mobile elements of bacteria inhabiting environments unaffected by human activities is needed. Bacteria preserved in permafrost sediments over periods of geological time scale offer a unique opportunity to study diversity and structure of ancient bacterial mobilome existing before the anthropogenic impact. In the present study three ancient mercury-resistant *Acinetobacter lwoffii* strains, isolated from East-Siberian permafrost sediments (dated to 15.000-30.000 years and 2-3 million years B.P.) (Kholodii et al. 2004), were investigated with respect to their plasmidome and heavy metal resistance profile.

MATERIALS AND METHODS

Common DNA manipulation methods were performed. Complete genomic sequences were obtained using MID-tagged shotgun method. PCR and sequencing was used to read physical gaps between assembled contigs.

Plasmid nucleotide sequences were analysed using Unipro UGENE, GeneMark.hmm prokaryotic and BLAST programs, IS-Finder and UniProt databases.

Acinetobacter lwoffii strains ED23-35, ED45-23 and VS15 were grown in TSA (Oxoid, GB) medium at 30 °C. Antibiotics were added at the following concentrations (μg/mL): ampicillin (200), streptomycin (100), spectinomycin (100), tetracyclin (10) and rifampicin (30).The spectrum of heavy metal ions resistance was determined using $CdCl_2xH_2O$, $NiSO_4x7H_2O$; $CoCl_2x6H_2O$, $K_2Cr_2O_7$, $ZnSO_4$.

RESULTS

We revealed the presence of 8 circular replicons in ED23-35, 9 in ED45-23 and 2 in VS15 ranging in size from approx. 4 kb to 287 kb. Each strain harboured one large plasmid: pALWED1.1 (287 kb) from ED23-35, pALWED2.2 (190 kb) in ED45-23 and pALWVS1.1 (130 kb) in VS15. These plasmids contained 12 to 18 IS-elements of IS1, IS4, IS5, IS6 and IS66 families. Large plasmids also carried genes homologous to mercury, cobalt, zinc, cadmium, chrome and arsenical compounds resistance operons, several copies of copper and iron intake regulating operons, urea degrading operon and tetracycline resistance genes (tetA-tetR). Smaller plasmids also carried chrome and urea resistance operons and aadA streptomycin/spectinomycin resistance gene. All identified plasmids encoded either conjugation or mobilisation systems.

Preliminary analysis of heavy metal resistance spectrum revealed that strains were resistant to 0.4mM Cd^{2+}, 0.4mM Co^{2+}, 3mM Cr^{6+}, and 3mM Ni^{2+} in addition to 0.03mM of Hg^{2+} compounds. These strains were also resistant to ampicillin, streptomycin/spectinomycin, but not tetracycline.

DISCUSSION

The large number of plasmids potentially capable of transferring horizontally and the abundance of heavy metal resistance genes encoded on them indicate the need for adaptive traits even in pristine ecosystems unaffected by human activities. Physical co-localisation of heavy metal resistance operons often flanked by IS-elements makes discovered replicons ideal for the horizontal dissemination of various resistance phenotypes. Plasmid-encoded genes conferring resistance to other toxic compounds, such as urea and active forms of oxygen, as well as genes of various transmembrane transportation systems of complex compounds, indicate the need to adapt to harsh and nutrient-poor permafrost conditions. Thus, studying ancient *Acinetobacter* strains isolated from Siberian permafrost, we demonstrated the diversity of plasmids and determined their adaptive value.

KEYWORDS: PERMAFROST, ACINETOBACTER LWOFFII, PLASMIDOME, HEAVY METAL RESISTANCE, BACTERIAL ADAPTATION

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Poster C-07

GROWTH REQUIREMENTS OF *Stichococcus* sp. STRAINS ISOLATED FROM RHODOPE MOUNTAIN, BULGARIA

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INTRODUCTION

Bulgarian climate and relief assign favorable conditions for the growth and development of snow algae. The snow fields in Rhodope Mountain represent one of the recently explored areas in a mountain massif higher than 2000 meters (Lukavský et Cepák 2014).

Stichococcus is one of the common algal species found in such an environments and its eco-physiology has been object of number of previous experiments (Kvíderová et Lukavský 2005).

In the present study the growth requirements of two strains of *Stichococcus* sp. (Rhod 42 and Rhod 48), isolated from snow surface at Rhodope Mountain in Bulgaria, were studied and compared to other two strains, *Stichococcus minutus* Kastovska 2002/19 and *Stichococcus* sp. Hindák 1963/85, deposited in CCALA – Trebon.

MATERIALS AND METHODS

The fourth strains were grown for 23 days at different conditions using the „crossed gradients" method (Kvíderová et Lukavský 2001). Nutrition medium Zehnder - „Z" (Staub, 1961) was used in three different concentrations: 2Z, Z, 0.5Z. Irradiance by LED was maintained in the range 160 - 560 µmol m^{-2} s^{-1}. The temperature range was 4 - 28 °C.

RESULTS AND DISCUSSION

After 23 days of cultivating it was found that the strains grow at wide irradiance interval and the main factors that affected the growth are temperature and the concentration of the nutrition medium. The strains *Stichococcus minutus* Kastovska 2002/19 and *Stichococcus* sp. Hindák 1963/85 showed similar behavior. The optimum temperature of the both strains was estimated at around 21 °C. They have their higher growth at 2Z concentrated medium and it decreases proportionally to the concentration of the medium.

The optimal temperature of strain *Stichococcus* sp. Rhod 48 was estimated at around 16 °C. Comparison to the previously described strains *Stichococcus* sp. Rhod 48 strain showed the highest growth at lowest concentration of the medium. Considering that Z nutrition medium is relatively pure of nutrients it could be assume that Rhod 48 belongs to the groups of oligotrophic organisms.

The other Bulgarian strain, Rhod 42 showed kind different behavior to the growth conditions. Regardless, it was isolated from similar localities to *Stichococcus* sp. Rhod 48, *Stichococcus* sp. Rhod 42 requires lower temperature (less than 14 °C) and could be considered as psychrophilic alga. Furthermore, *Stichococcus* sp. Rhod 42 changes its temperature optimum and optimal irradiance range, according to the concentration of the nutrition medium.

After 23 day of cultivating the highest biomass was received from *Stichococcus minutus* Kastovska 2002/19 (4.27 g dm^{-3}). *Stichococcus* sp. Rhod 42 showed lowest growth rate due to its very long lag-phase.

The comparing according to the growth requirements of the studied strains is shown in Fig. 1.

After comparing of studied *Stichococcus* sp. strains, according to their growth requirements it could be assume that *Stichococcus minutus* Kastovska 2002/19 and *Stichococcus* sp. Hindák 1963/85 have relative requirements to temperature and nutrient. The strain *Stichococcus* sp. Rhod 48 is similar to them, but it requires lower concentration of the nutrients. In contrast *Stichococcus* sp. Rhod 42 differs from the other studied strains. The future studies will show its physiological and biochemical characteristics.

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KEYWORDS: BULGARIAN MOUNTAINS, SNOW ALGAE, *Stichococcus* sp.

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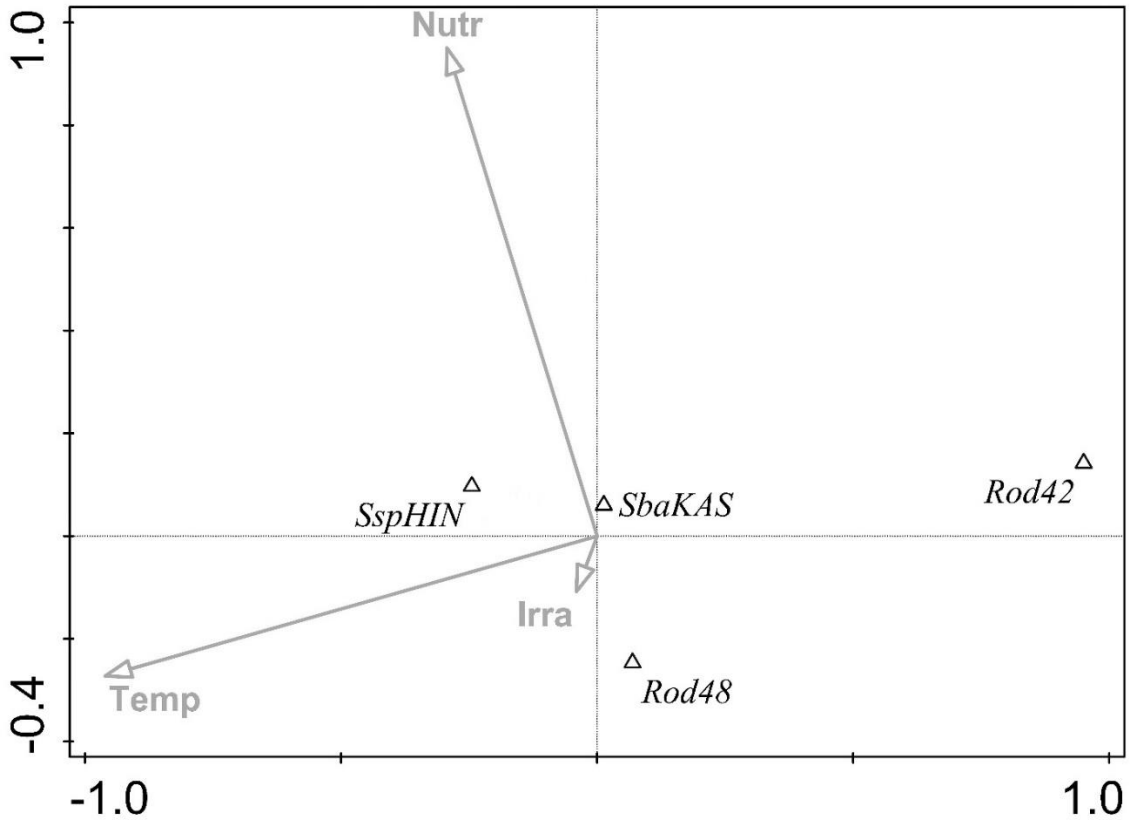

Fig. 1. Comparing of studied *Stichococcus* sp. strains, according to their growth requirements

Poster C-08

PROTEOMICS AND GENETICS OF HALOARCHAEA FROM DEEP LAKE, ANTARCTICA

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Antarctic halophilic archaea (haloarchaea) dominate life in Deep Lake, Vestfold Hills, East Antarctica (DeMaere et al. 2013). Deep Lake is a 3,500 year old marine-derived system that is so saline the lake remains liquid, even when temperatures drop to –20°C. Genomic and metagenomic analyses showed that *Halorubrum lacusprofundi* represents ~ 10% of the Deep Lake community (DeMaere et al. 2013). Its relative abundance has been linked to differential abilities to utilize nutrients and other physiological distinctions (*i.e.* niche adaptation) (DeMaere et al. 2013, Williams et al. 2014). Niche adaptation is an important selection pressure maintaining speciation that counteracts high levels of haloarchaea intergenera gene exchange (DeMaere et al. 2013).

To investigate ecologically relevant mechanisms of growth, survival and speciation in the lake, quantitative proteomics (iTRAQ) was developed, and pathways and cellular processes involved in adaptation to the cold, biofilm/aggregate formation and metabolism investigated. *Hrr. lacusprofundi* produces extracellular polymeric substances, aggregates and biofilms in response to growth temperature (Fröls etal. 2012, Reid et al. 2006), specific substrates, and growth phase. The formation of aggregates and biofilms may promote gene exchange. Using 8-plex iTRAQ labelling, proteins important for cold adaptation, aggregation, and nutrient utilization were determined for *Hrr. lacusprofundi*. For example, compared to planktonic cells, *Hrr. lacusprofundi* biofilms were characterized by increased carbon fixation and synthesis of S-layer glycoproteins and extracellular polymeric substances, consistent with forming biofilm structures, while also demonstrating responses to nitrogen limitation and oxidative stress and reducing global protein synthesis.

Gene transfer and gene knockout systems were developed for *Hrr. lacusprofundi* in order to probe the role of specific genes. The *Hrr. lacusprofundi* genome lacks urease genes and cells are urease negative, yet cells grow with urea as a sole nitrogen source. From proteomic assessments, the acetamidase Hlac_2285 was found to be up-regulated during growth on urea (compared to ammonia). To study the role of this acetamidase in urea utilization, Hlac_2285 was inactivated. The mutant showed similar growth to the wild-type strain when urea was the sole nitrogen source. Ongoing work is evaluating the effect of inactivating a second acetamidase gene (Hlac_2016). When complete, these genetic assessments will clarify the role of acetamidase genes in urea utilization.

The ability to perform gene transfer and construct knockouts provides a major advance in being able to probe the molecular mechanisms of adaptation in *Hrr. lacusprofundi*, and combines well with the development of proteomic methods for assessing regulation of global gene expression in important lake species.

KEYWORDS: PROTEOMICS; BIOFILMS; ADAPTATION; GENETIC MANIPULATION; *ARCHAEA*

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Poster C-09

PHOTOSYNTHETIC AND GENOMIC RESPONSES OF *Chlorella* SPECIES FROM DIFFERENT GEOGRAPHICAL REGIONS TO ARTIFICIAL ULTRAVIOLET RADIATION (UVR) STRESS

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Changes in photosynthesis efficiency and heat dissipation can be used as an early stress indicator in phototrophs. In our study, chlorophyll fluorescence was measured by Pulse-Amplitude-Modulation Fluorometer (PAM) to determine the photosynthetic performances of Antarctic *Chlorella* (UMACC 237), Arctic *Chlorella* (UMACC 263), temperate *Chlorella* (UMACC 248) and tropical *Chlorella* (UMACC 001) in response to artificial ultraviolet radiation (UVR) stress. The primary objective of this study is to investigate the photosynthetic and genomic responses of *Chlorella* from different geographical regions towards artificial ultraviolet radiation stress.

Three types of light treatment were conducted in a continuous six hours duration: (i) PAR (control), (ii) PAR+UVA (UVA stress) and (iii) PAR+UVA+UVB (UVB stress). Few photosynthetic parameters were determined by the PAM. UVB radiation caused significantly photosynthesis stress as compared to the UVA-treated and the controlled samples.

For the genomic response study, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was conducted to identify the change in nucleotide sequence between the controlled sample and the samples subjected to UVB and UVA treatments. A few targeted markers that coded for photosynthesis-related gene was first amplified by specific primer and then digested with 4 base pairs cutter. DNA fingerprint profiles of control and treated samples will be visualized and analyzed by gel electrophoresis.

KEYWORDS: *Chlorella*, ULTRAVIOLET RADIATION, PULSE AMPLITUDE MODULATED FLUOROMETER, PCR-RFLP

Poster C-10

CHANGES IN STRUCTURE, ACTIVITY AND METABOLIC PROCESSES OF MICROORGANISMS IN THAWING PERMAFROST SOILS FROM SVALBARD

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About half of the global belowground organic carbon pool is stored in Arctic permafrost (Tarnocai et al. 2009). Changes in a warming Arctic will lead to increased microbial activity in permafrost soils, which will alter the Arctic carbon budget (Schuur et al. 2009, Mackelprang et al. 2011). With thawing permafrost, the rate of decomposition of stored organic carbon increases and elevated levels of greenhouse gases are released into the atmosphere. In this study we aim to designate dominant microbial groups and their potential metabolic activity and changes of key processes due to accelerated thaw in permafrost cores from Svalbard. A specific focus is put to the degradation potential of organic matter in distinctive different layers of the permafrost soil.

Soil cores (2 meters) have been obtained from a characteristic low-centered ice-wedge polygon site in the valley Adventdalen on the island Spitsbergen in Svalbard (15.9248E, 78.186N) during Arctic summer. They were subjected to a CT scan, taken every 1 cm, which generated a structural profile and showed the transition zone between active and permafrost layer. Subsamples covering all three zones, were selected for DNA extraction. The extracted community DNA has been used as template in 16S rRNA amplification in combination with massive parallel sequencing to obtain a description of the microbial community along the core. Additionally, metagenomic analyses were performed to indicate the key functional processes in the distinctive layers. To identify both the changes in community structure and gene abundance differences due to thaw, the same permafrost samples were used in incubation experiments where microbial activity was measured. Gas fluxes of CO_2; CH_4 and N_2O were monitored over the course from hours up to several days while incubating under temperatures occurring during the thawing season (4-6°C) and DNA/RNA were extracted after the incubation was terminated.

First results point towards characteristic differences in the microbial community structure originating from the active, transition and permafrost zones. In the active layer the predominant groups were Acidobacteria, Actinobacteria, Proteobacteria and Verrucomicrobia. Then in the transition zone the Actinobacteria take over and totally dominate the permafrost layer. Interestingly, overwhelming majority of Actinobacteria belongs to *Intrasporangiaceae* (family), different than previously reported. In general most of the genomes both in the active layer as well as in the permafrost are composed of C-cycling genes. Svalbard permafrost sample is unique in somewhat specialization into *Intrasporangiaceae* and overall low abundance of methanogens. Thawing of the permafrost indicates a rapid release of stored CO_2; CH_4 and N_2O and community changes due to increased degradation of organic matter. Interestingly, the different layers show diverse responses to thawing and alarmingly the upper and deep permafrost soils show the highest CO_2 production rates. Together these results help to better understand the changes of thawing permafrost on the microbial level.

Microbes are recognized as the main drivers of the processes that determine the balance of carbon storage and release in the Arctic. Our results indicate that increasing temperatures may have an especially strong effect on the community structure and degradation potential in the deeper permafrost zones where vast amounts of labile carbon are stored.

KEYWORDS: PERMAFROST; MICROBES; METHANE; CARBON CYCLE

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Poster C-11

PROSPECTION AND DESICCATION TOLERANCE OF POLAR MICROORGANIMS

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Liquid water is being considered the key factor for the existance of Life on Earth and abroad. Earth's polar regions have remarcably low humidity rates due to sub-zero temperatures throughout the year. Still, microbial communities in those regions are diverse, even in less dynamic ecossystems such as the permafrost. This research aims to isolate cold and desiccation tolerant bacteria from Arctic and Antarctic soils and characterize these strains to desiccation tolerance. Although studies comparing the microbiology of the poles have been made, comparative studies with desiccation-tolerant strains from Polar regions were never reported.

Antarctic samples were collected within the Brazilian Antarctic Program (PROANTAR) during the International Polar Year. Soil samples were collected from multiple locations in Deception Island (an active volcano) and from soils exposed by the Baranowski Glacier retreat (King George Island). Arctic samples were collected from a three-strata soil core (Active layer, Middle Layer, Permafrost) near the McGill High Arctic Research Station, Axel Heiberg Island, Canada. Bacterial strains were isolated after treating the soil samples with ≥ 99.8% chloroform for 30 minutes, in order to mimic the pressure exerted by the absence of water for prolonged periods of time (Narvaez-Reinaldo, Barba et al., 2010). Chloroform treated soils were inoculated in R2A and TSA media, and incubated at 30 °C or 0 °C until the appearance of colonies. Isolates were identified with partial 16S rRNA gene sequencing. The desiccation tolerance was examined by desiccating the isolates in an exsiccator during 0, 1, 5 and 50 days in R2B 10% medium or water. Strains of *Deinococcus radiodurans* and *Escherichia coli* were used as a positive and negative control for desiccation tolerance, respectively. In addition, strains from Antarctica (*Exiguobacterium antarcticum*) and Arctic (*Planococcus halocryophilus*) were also tested as another mean of comparison.

All chloroform treated soil samples showed colonies, although not in all incubation temperatures. From Deception Island, a region of volcanic activity, we only obtained colonies at 30° C. In the Antarctic soils recently exposed by glacier retreat (1-2 years) colonies appeared only at 0 °C, while in older soils (30-35 years) colonies grew only at 30 °C. From the Arctic core, we obtained colonies from both temperatures in all strata. A total of 57 strains were identified by 16S rRNA gene sequencing, belonging to the Phyla: *Actinobacteria* (1 strain, Deception Island), *Proteobacteria* (9 strains, Deception Island) and *Firmicutes* (47 strains from all the sampling sites). From these, 24 strains were characterized by its desiccation tolerance. All strains presented a higher survival rate when desiccated at R2B medium. Under this condition, 5 strains showed less than 10-fold reduction in viability after 50 days of desiccation (1 from Phylum Actinobacteria, Family Micrococcaceae; 4 from Phylum Firmicutes, Family Paenibacillaceae), surpassing the positive control ability to withstand drought stress. *E. antarcticum* and *P. halocryophilus* suffered 10 to 100-fold viability reduction after 5 days, after 50 days of treatment the results were similar to the negative control. Interestingly, we have seen notable differences in desiccation tolerance among microorganisms from closely related strains of the same genus. Further studies of these strains may provide correlation between specific adaptations and anhydrobiosis survival.

KEYWORDS: ANTARCTICA; ARCTIC; DESICCATION; TOLERANCE; PSYCHROTROPHS; BACTERIA

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Poster C-12

THE MOLECULAR BASIC OF THERMOSTABILITY OF COLDACTIVE ESTERASE FROM PSYCHROTROPHIC BACTERIUM *Psychrobacter cryohalolentis* K5^T

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The permafrost regions occupy about 25% of the Northern Hemisphere's terrestrial surface, and more than 50% of that of Russia. Permanently frozen sediments pose unique challenges to its resident biota: low temperatures and small amounts of liquid water, limited access to nutrients. It was shown previously, that permafrost contains the unique microbial community adapted to these specific conditions. It is inhabited by bacteria, green algae, yeasts, mycelial fungi. One of survival strategies for these microorganisms is the expression of cold-active enzymes. Esterase EstPc is a recently discovered lipolytic enzyme from permafrost bacterium *P. cryohalolentis* K5^T, possessing relatively high thermal stability and elevated enzymatic activity at temperatures near 0°C. To unravel the molecular mechanisms underlying such unusual properties of EstPc we have utilized the site-directed mutagenesis. We have obtained and studied mutant variants of EstPc containing substitutions of amino acid residues near the catalytic triad's residues and deletions of various regions. Mutant proteins with substitutions near the catalytic residues histidine and aspartate demonstrate optimum activity shifted to lower temperatures, and low thermal stability. The deletion variant which lacks the N-terminal alpha-helix has reduced activity and a temperature optimum within a narrow temperature range but exhibits an elevated resistance to heating at high temperatures. Mutant proteins with deletions of various length within NC-loop exhibited a lower temperature activity optimum and narrow range of substrate specificity and temperature dependence. Overall, our data demonstrate the important contribution of these regions to the thermal stability of EstPc, since their changes or deletions result in the disappearance of the characteristic properties of the protein.

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KEYWORDS: COLDACTIVE ENZYMES, ESTERASE, PERMAFROST, THERMOSTABILITY ER-CASE LETTERS

Poster C-13

A SINGLE CELL VIEW OF THE GROWTH OF THE ANAEROBIC BACTERIUM *Clostridium psychrophilum* AT SUBZERO TEMPERATURES

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INTRODUCTION

Anaerobic psychrophilic microorganisms have received so far little attention, thus not much is known about their capability to adapt and grow at subzero temperatures. However, this type of extremophiles is of special importance with respect to the functioning of cold ecosystems on Earth, potential biotechnological applications, and also for the search of life on extraterrestrial icy bodies and planets.

The genus *Clostridium* accounts at present for the largest number (about 10) of psychrotolerant and psychrophilic anaerobic organisms, and includes strains isolated from permafrost (*C. algoriphilum*, *C. tagluense*), cold sediments (*C. vincentii*) and microbial mats in Antarctica (*C. psycrhophilum*, *C. frigoris*, *C. lacusfryxellense*, *C. bowmanii*) and also chilled meat (*C. gasigenes, C. estertheticum*) (reviewed by Finster 2008). The eco-physiology of such organisms under frozen conditions, including the response and adaptation mechanisms, remain largely unexplored.

In our work we set out to determine the lowest temperature that supports cell replication as well as metabolic activity in *Clostridium psychrophilum*. In parallel, we also looked into cold-adaptive strategies at both the morphological, structural and metabolic level, which make life under such extreme conditions possible.

METHODS AND MATERIALS

C. psychrophilum, isolated from the Antarctic lake Fryxell (Spring et al. 2003), was used as model organism for this study. Cell enumeration experiments using fluorescent dyes were combined with stable isotope probing techniques. Metabolic activity was assessed in terms of assimilation of ^{13}C-labelled carbon substrates both in the bulk biomass (EA-IRMS) and in single cells using nanoSIMS. Metabolic products were also identified and quantified by SPME-GCMS. To identify temperature-related morphological changes, electron microscopy was performed in parallel.

RESULTS

The lowest temperature at which cell replication could be measured directly at the population level is -5°C. At -10 and -15°C, the metabolic level, and thus the growth rates, were sensibly reduced, and could be measured only at the level of single cells based on the turnover of the ^{13}C-labelled carbon source. Various strategies to respond to the freeze-stress could be observed: some cells could be imaged while undergoing division at -15°C, some showed filamentous growth without dividing, and some others were found accumulating the carbon source in intracellular storage granules (Perfumo et al. 2014).

DISCUSSION

C. psychrophilum was demonstrated to be active at temperatures as low as -15°C. Cell heterogeneity was predominant phenomenon, which lead to a diversified, possibly enhanced, range of adaptive strategies to such extreme environmental conditions.

KEYWORDS: CRYO-ADAPTATION; SINGLE CELLS; STABLE ISOTOPE PROBING; FILAMENTOUS GROWTH; CARBON STORAGE

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Poster C-14

NEW AUTOTRANSPORTER FROM *Psychrobacter cryohalolentis* K5^T: CHARACTERIZATION AND CONSTRUCTION OF CELL SURFACE DISPLAY SYSTEM

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The autotransporter (AT) family includes outer membrane proteins from Gram-negative bacteria consisting of N-terminal passenger and C-terminal translocator domains. They have diverse functions including adhesins, toxins, enzymes etc. In our previous work we have initiated the study of the lipolytic system of a Gram-negative bacterium *Psychrobacter cryohalolentis* K5^T isolated from cryopeg – a lense of overcooled water brine within Siberian permafrost soil (Bakermans et al. 2006). The gene coding for a potential AT protein (AT877) was annotated in *P. cryohalolentis* K5^T genome as a putative member of the GDSL lipase family. This protein was overexpressed in *Escherichia coli* cells and localized in the outer membrane fraction. AT877 displayed maximum hydrolytic activity on medium-chain *p*-nitrophenyl esters (C8-C10) at 50ºC. It was resistant to the presence of several metal ions, organic solvents and detergents.

To evaluate applicability of AT877 for the cell surface display of heterologous passengers we have fused the coding sequences of its α-helical linker and translocator domain with that of esterase EstPc from *P. cryohalolentis* K5^T(Novototskaya-Vlasova, K. et al. 2012). Expression of the hybrid EstPc877 protein produced a new cold-active esterase with high whole cell activity at 5-30°C (Petrovskaya 2015). Cell fractionation studies and esterase activity measurements demonstrated that EstPc passenger with a temperature optimum at 15-25°C and a substrate preference toward *p*-nitrophenyl butyrate (C4) was successfully displayed at the surface of the induced cells.

In the subsequent experiments we have demonstrated the applicability of the developed system for the display of different heterologous passengers including tenth type III domain of human fibronectin and fluorescent protein mCherry. As it was shown by whole cell ELISA and confocal microscopy, these proteins were successfully displayed at the surface of the recombinant *E. coli* strains. Obtained results prove that the presence of the AT877 α-helical linker and the translocator domain is sufficient for the targeting of various passenger proteins to the surface of *E. coli* cells. They represent a new example of the biotechnologically relevant enzyme from the unique microbial community of permafrost and a potential basis for the construction of a new cell-surface display platform.

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KEYWORDS: PERMAFROST, AUTOTRANSPORTER, LIPASE, *Psychrobacter cryohalolentis* K5^T, CELL-SURFACE DISPLAY

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Poster C-15

DESICCATION TOLERANCE AND FATTY ACID COMPOSITION OF POLAR GREEN ALGAE *Zygnema* spp. (ZYGNEMATOPHYCEAE, STREPTOPHYTA)

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INTRODUCTION

Conjugating green algae (Zygnematophyceae, Streptophyta) produce high amounts of biomass in polar hydro-terrestrial environment. Such habitats are usually shallow with only limited supply of liquid water from melting snow and active permafrost layer. Thus, the algae are subject to desiccation during the vegetation season. Production of stationary phase like cells, so called pre-akinetes has been recently reported from alpine and polar *Zygnema* spp. (Herburger et al. 2015, Pichrtová et al. 2014a). Moreover, formation of the pre-akinetes was induced by starving in laboratory conditions (Pichrtová et al. 2014b). Here, recent results of desiccation tolerance studies on *Zygnema* spp. will be presented, focusing on the role of pre-akinetes and their different ultrastructure and biochemical composition in comparison to vegetative cells.

METHODS AND MATERIALS

Three *Zygnema* sp. strains were selected for the experiments, one (B) from the Arctic and two (C, E) from the Antarctica. Both fresh cultures and cultures consisting of pre-akinetes were desiccated over saturated KCl solutions (RH 86 %). The effective quantum yield of photosystem II was regularly measured during dehydration and subsequent rehydration and the ultrastructure of the cells was investigated by transmission electron microscopy. Finally, the fatty acid composition was studied by FAME analysis.

RESULTS AND DISCUSSION

Only cultures consisting of pre-akinetes were able to survive desiccation. The pre-akinetes were less physiologically active than vegetative cells, their chloroplasts had reduced lobes and the electron microscopy revealed large accumulations of lipids. Moreover, significant differences in the fatty acid composition between young and pre-akinete cultures were detected, the most abundant fatty acids in pre-akinetes were oleic and linoleic acid. However, hardly any impact of the desiccation and recovery on fatty acid composition was revealed. The results indicate that naturally hardened pre-akinetes play a key role in desiccation tolerance and survival of polar *Zygnema* spp. while the production of other types of specialized cells is largely suppressed.

KEYWORDS: DESICCATION TOLERANCE, GREEN ALGAE, LIPIDS

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Poster C-16

LIGHT AND TEMPERATURE DEPENDENCE OF PHOTOSYNTHESIS IN CHLAMYDOMONADS ISOLATED FROM SNOW

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Snow algae have been reported from polar and mountain regions worldwide. Their adaptation to extreme conditions of life in the snow column and their ability to perform photosynthesis at rates that are comparable to aquatic microalgae and land plants of temperate regions have been topics of only a handful of studies, the latter being mainly carried out on cysts collected from field samples. Here we describe the photosynthetic performance of five strains of snow algae belonging to the family *Chlamydomonadaceae* (Chlorophyta) that were isolated from snow above the timberline: *Ladove* (Slovakia, strain CCALA 970), *Abisco* (Sweden), *Furka* (Switzerland), *Gossenköllensee* (Austria) and *Tromsø* (Norway). The strains were perpetuated in their flagellate stages by continuous growth in air-bubbled liquid BBM medium at 2 °C under permanent illumination of 150 µmol photons m^2 s^{-1}, provided by LED light sources. Cells were diluted to 2 µg Chl ml^{-1} and 2 ml of culture was transferred to a magnetically stirred closed thermostated cuvette. The oxygen evolution was measured using a Clark-type concentration electrode at 2 °C and at increments of 5 °C in the range from 5 to 35 °C, at light intensities from 6 to 13300 µmol photons m^2 s^{-1}. Analyses were carried out as described previously (Lukeš et al. 2014). All experiments were carried out in triplicates.

Maximum photosynthetic oxygen-evolving activity from H_2O to CO_2 (P_m) showed a linear increase with rising temperature, peaking at 20° C (*Tromsø*), 25° C (*Ladové*, *Furka*, *Gossenköllensee*) or 30 °C (*Abisco*) with 180–895 µmol O_2 mg Chl^{-1} h^{-1}. A further increase in temperature usually led to a sharp decline of activity. In particular, the highest gross P_m was exhibited by *Tromsø* (895 µmol O_2 mg Chl^{-1} h^{-1}) whereas other strains reached it at two-four times lower level. Generally, net oxygen production was detected in a wide range of irradiances (138–4250 µmol m^{-2} s^{-1}) and temperatures (2–30 °C; only 2–25 °C in *Gossenköllensee*, up to 35 °C in *Furka*). At low temperatures of 2 and 5 °C, the compensation point was found at the irradiance of 16 µmol m^{-2} s^{-1} in *Abisco*, *Furka* and *Ladove*, while *Gossenköllensee* and *Tromsø* had their compensation point at 50 µmol m^{-2} s^{-1}. Photoinhibition always occurred at very high irradiance levels (≥ 4250 µmol m^{-2} s^{-1}). The initial slope of P/I curves α, reflecting light-harvesting and photosynthetic energy conversion efficiency, showed a linear increase with increasing temperature from 2 to 15 °C and from 10 to 20 °C in *Ladove* and *Tromsø* strains respectively. In contrast, α peaked at 2 °C in *Furka* and *Gossenköllensee* and at 5 °C in *Abisco*. Levels of α in *Ladove* and *Tromsø* were approximately 1.5 higher than in *Abisco*, *Furka* and *Gossenköllensee*. In all cases, the lowest value for α were observed for the highest temperature of 35 °C.

In summary, our data showed that short living flagellate stages exhibited a comparable or slightly lower photosynthetic rate than the immotile long living cysts did (Remias et al. 2005) while utilizing very high irradiances which are regarded lethal for the majority of algae species. Photosynthesis of all strains of snow algae was more effective at higher temperatures (P_{max}; 20–30 °C) than expected for the snow environment. We have shown earlier that the *Ladove* strain had a significantly higher oxygen-evolving activity at temperatures from 2 to 20 °C in comparison with mesophilic *Chlamydomonas reinhardtii* grown at 24 °C (Lukeš et al. 2014). The new data suggests that the snow algal photosynthesis is that of a mesophilic organisms encased in a cell well adapted to a low temperature environment.

KEYWORDS: ALGAE, SNOW, PHYSIOLOGY, PHOTOSYNTHESIS, OXYGEN EVOLUTION

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Poster C-17

SIGNIFICANT CYTOLOGICAL AND PHYSIOLOGICAL DIFFERENCES BETWEEN TWO GREEN ALGAE CAUSING RED SNOW IN THE ALPS

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Blooms of microalgae cause tinted snow in mountainous and polar regions during summer. They are important primary producers in seasonal ecosystems of melting snowbanks and glacier surfaces (Lutz et al. 2015). Snow algae are regarded as extremophiles due to harsh abiotic conditions in their habitat like exposure to extreme VIS and UV irradiation, permanently low temperatures, a short vegetation period or frequent freeze-thaw-cycles. Thus, special mechanisms of adaptation are expected in terms of cytology, life cycle and physiology (Remias 2012).
Red snow caused by Chlamydomonadalean spores, which are weak in morphologic details, is probably the most common phenomenon. The cells are traditionally associated with the collective species *Chlamydomonas* cf. *nivalis*. Molecular studies using 18S rRNA gene have shown that different taxa cause red blooms, which however are not individually described as species yet (Leya 2004). Moreover, no details are known so far if cytology and physiology of these spores are identical, or if different strategies against the harsh environment have evolved. We compare field samples of two distinct populations from Austria, one typically occurring in seasonal snowfields above timberline (species A, resembling *Chlamydomonas* cf. *nivalis*), the other living in slush at the ice cover of a high alpine lake (species B, resembling *Chlainomonas rubra*).
The methods include light- and electron microscopy, photosynthesis and respiration measurements and a chemotaxonomic characterization of secondary astaxanthin derivatives using high performance liquid chromatography in combination with mass spectrometry and atmospheric pressure chemical ionization (HPLC-MS/APCI).
The results show significant differences in cytoarchitecture and metabolism. While spores of species A always possesses a single axial chloroplast surrounded by “shading” lipid globules containing astaxanthin pigments, species B has numerous small disc-like plastids, which apparently can move within the cytoplasm from a parietal, fully exposed position to a protected central one, then also mimicking a single massive chloroplast in bright field light microscopy. As a consequence, species B seems to be able to adapt to varying light conditions, which may change from low irradiation in deeper snow layers to high light conditions at the surface.
In a chemotaxonomical approach, the individual astaxanthin-esters of both species were compared in terms of chromatographic retention time and molecular mass (Fig. 1). A and B share signatures, but in majority have individual patterns of astaxanthin derivatives. Using a similar method, Řezanka et al. (2013) found differences between Alpine and Bulgarian populations. This demonstrates that astaxanthin is esterified with distinctive fatty acids residues, making a differentiation by analytic methods feasible.

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KEYWORDS: SNOW ALGAE, PIGMENTS, ULTRASTRUCTURE, CHEMOSYSTEMATICS

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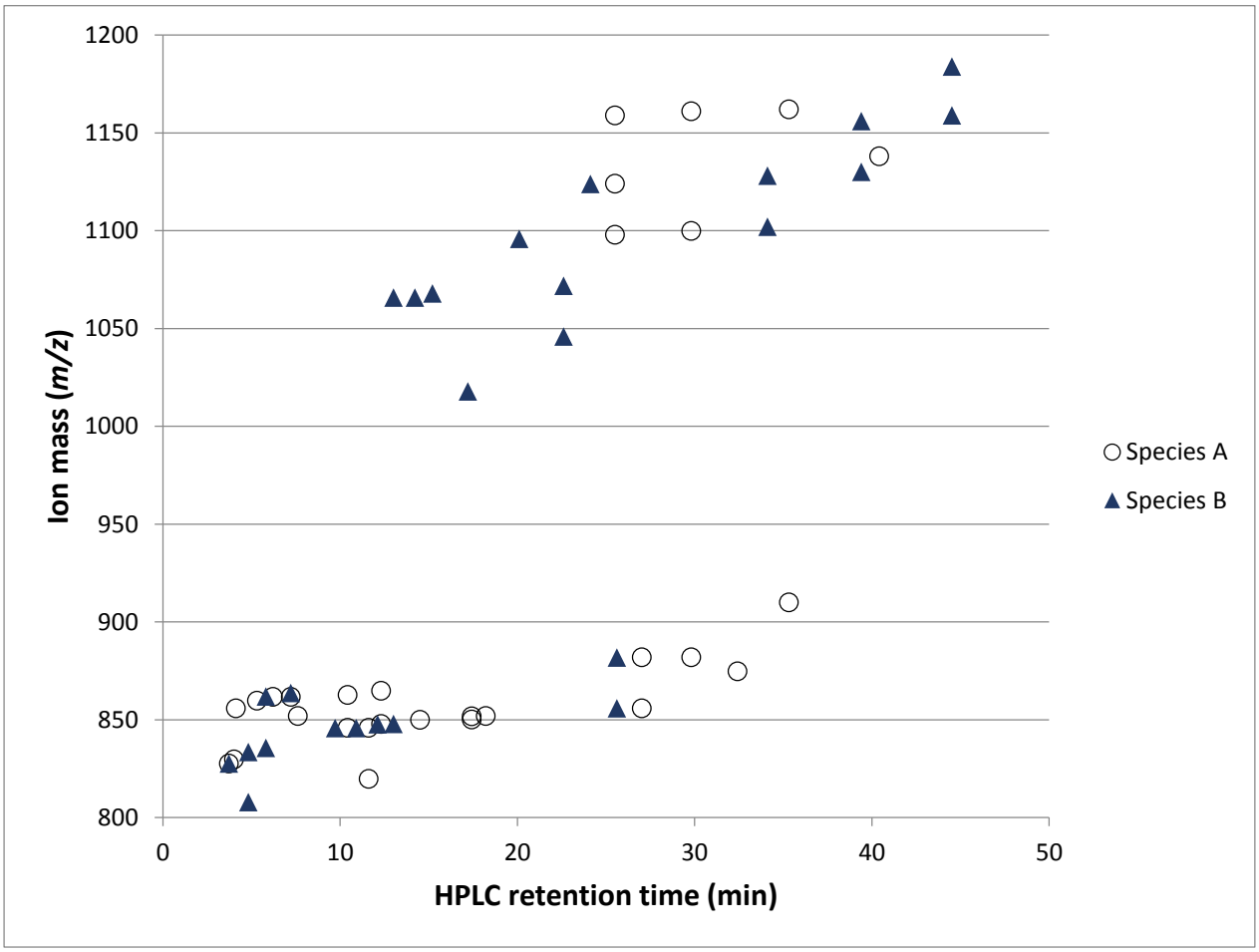

Fig. 1. Secondary red pigments (astaxanthin-esters) of two morphologic similar snow algal spores (Chlamydomonadalean species A/B) were analyzed by HPLC (giving retention time, roughly depending on compound polarity) and MS (giving ion mass).

Poster C-18

DIFFERENTIAL UTILIZATION PATTERNS OF DISSOLVED ORGANIC PHOSPHORUS COMPOUNDS BY HETEROTROPHIC PLANKTONIC BACTERIA

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Although phosphorus limitation is common in freshwaters and dissolved organic phosphorus (DOP) can be taken up by bacteria to meet their P requirements, very little is known about the use of different DOP compounds by individual bacterial groups. Here, we studied the uptake of three different DOP compounds and determined whether uptake preferences followed concentration-dependent patterns, in two mountain lakes differing in their trophic status. We assessed ATP, glucose-6-phosphate, and glycerol-3-phosphate uptake patterns among individual bacterial groups by means of microautoradiography combined with catalysed-reporter-deposition fluorescence-*in-situ*-hybridization. Additionally, we determined bulk uptake rates by the whole microbial community to estimate the kinetic parameters v_{max} and K_M. Our single-cell approach showed that in the subalpine lake, bacteria took up glucose-6-phosphate (mean: 39.85±6.79% of eubacterial cells taking up the substrate) preferentially, whereas all three substrates were taken up to a similar extent in the alpine lake (mean 29.66±4.30%). The pronounced uptake of glucose-6-phosphate in the subalpine lake was mirrored at the individual group level, whereas in the alpine lake bacterial groups exhibited distinct uptake patterns. *Betaproteobacteria* and its R-BT cluster were overrepresented in the uptake of glucose-6-phosphate and glycerol-3-phosphate in both lakes, but this trend was more pronounced in the alpine than in the subalpine lake. In contrast, AcI *Actinobacteria* tended to be underrepresented in the uptake of those substrates and they were also weakly represented in ATP incorporation. *Alphaproteobacteria* and *Cytophaga-Flavobacteria* exhibited variable DOP uptake patterns which differed between lakes and depths (epilimnion, hypolimnion). The substrate affinity constant K_M was lowest for ATP in the alpine lake, whereas in the subalpine lake, it was lowest for glucose-6-phosphate. Our results demonstrate that the three DOP compounds are readily used by the freshwater bacterioplankton and show that bacterial groups differ in their ability to take them up.

KEYWORDS: ATP/GLUCOSE-6-PHOSPHATE, GLYCEROL-3-PHOSPHATE, BACTERIAL GROUPS, MOUNTAIN LAKES

Poster C-19

ADAPTIVE FEATURES ENCODED WITHIN PLASMIDS OF ARCTIC AND ANTARCTIC *Psychrobacter* spp.

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INTRODUCTION

The major part of biosphere comprises environments where temperature never exceeds 5°C. Microorganisms living under these harsh and specific environmental conditions usually have to cope not only with low temperatures, but also nutrient limitation. Cold-adapted bacteria (psychrophiles and psychrotolerants) possess specific attributes that facilitate their growth in such extreme conditions. Ability to survive may be achieved by harboring mobile genetic elements, plasmids in particular, which are known to be the main players in horizontal gene transfer. Plasmids may encode various phenotypic modules which may enhance bacterial fitness.

Bacteria of the genus *Psychrobacter* are mostly isolated form cold environments. We analysed the mobilome of two psychrophilic *Psychrobacter* strains, DAB_AL32B and AH3, isolated from Arctic and Antarctic soil, respectively.

METHODS AND MATERIALS

We used common *in silico* methods for plasmid annotation (similarity searches, multiple sequence alignments, identification of protein conserved domains). Functionality of plasmid modules was examined in various members of *Proteobacteria* with the application of appropriate physiological and molecular tests. Methyltransferase genes were cloned, expressed and tested in *E. coli*.

RESULTS

We present results of structural and functional examination of thirteen plasmids isolated from *Psychrobacter* sp. DAB_AL32B and AH3 strains. Nucleotide sequence of these replicons was determined and analysed *in silico*. Three plasmids contained significant portion of accessory genetic information, including (i) type II restriction-modification systems, modules encoding enzymes involved in (ii) type 3 fimbriae synthesis and biofilm formation and (iii) glycine betaine, choline and carnitine transport and metabolism. Remaining replicons were found to be small cryptic plasmids.

Plasmid pP32BP2, originating from strain DAB_AL32B, carries three putative adaptive modules, MRK, BCC and CAI. Analysis of the MRK module revealed that this gene cluster significantly increases the host's ability to adhere, and therefore to form biofilms. Moreover, we present results of *in silico* and *in vivo* examination of BCC and CAI modules which may have role in carnitine utilization and protection against high osmolarity and low temperatures. Two plasmids of the AH3 strain (namely pA3H9 and pA3H10) carried type II restriction-modification genes with three methylases homologous to those associated with BfiI, EcoRI and MjaI systems. We determined the sequences methylated by these identified methylases.

DISCUSSION

We performed detailed analyses of plasmids of two *Psychrobacter* sp. strains. The combination of *in silico* and physiological analyses provided a valuable insight into the biology of psychrophilic bacteria belonging to the genus *Psychrobacter* and highlighted the role of mobilome in shaping bacterial fitness in relation to cold environment.

KEYWORDS: PSYCHROPHILE, PLASMID, PSYCHROBACTER, HOST ADAPTATION

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Poster C-20

STRUCTURAL AND FUNCTIONAL ANALYSIS OF WATER-BORNE SIGNALING PROTEIN PHEROMONES FROM THE BIPOLAR PROTIST CILIATE, *Euplotes petzi*

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INTRODUCTION

A major component of microbial polar communities is represented by protist ciliates (Petz 2005, Petz et al. 2007), which can be collected from their natural environment and stably adapted to proliferate in captivity, in a much easier way than other polar eukaryotic microbes. Among numerous Antarctic and Arctic *Euplotes* species presently cultivated in our laboratory, *E. petzi* has attracted particular interest because of its strict psychrophilic behavior (it does not tolerate temperatures above 8-9°C), and because of its capacity to constitutively secrete signaling protein pheromones.

METHODS AND MATERIAL

Pheromones have been isolated from genetically different *E. petzi* strains, and were characterized as small proteins of 32 amino acids. Structures were determined by Nuclear Magnetic Resonance spectroscopy (NMR).

RESULTS & DISCUSSION

The eight cysteines are located in strictly conserved positions, and predicted to form four intra-chain disulfide bridges. This high density of disulfide bridges would intuitively imply a quite compact globular molecular structure. However, this hypothesis is contradicted by preliminary structures calculated with NMR methods from purified protein solutions. The NMR structures show that the extension of regions devoid of regular secondary structures dominates over regions exhibiting regular □-helical organization. Considering that the structures of the analog pheromones from *Euplotes* species living in temperate waters is largely dominated by helical regions, our findings suggest a functional correlation of the *E. petzi* pheromone cold-adaptation with an increased flexibility of the molecular backbone and, hence, a decreased thermo-stability. This hypothesis is currently under verification by analyzing the unfolding and refolding properties of *E. petzi* pheromones when exposed to increased temperatures and to variations of other environmental parameters.

KEYWORDS: EVOLUTION; ADAPTATION; PROTEIN; BIOCHEMICAL CHARACTERIZATION; STRUCTURAL FLEXIBILITY

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Poster C-21

VIRAL-HOST INTERACTIONS IN GLACIAL ICE AND THEIR ADAPTIVE SIGNIFICANCE

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Cold environments have been proposed as hot spots for viral activity. Prokaryotic viruses have been found to be diverse, active and have broad host range in the cryosphere. Thus, the transfer of genetic material by viruses (transduction) could play an important role in the adaptation of microbial cells in these environments. However, exploration of the interaction between viruses and their hosts in cold environments is difficult in part due to the scarcity of viral sequences in the databases.

CRISPR (Clustered Interspaced Short Palindromic Repeats) systems retain viral sequences from past infections, and thus, could provide information about the role of viruses in the adaptation of the prokaryotic community in cold environments. Environments with relatively low temperatures were found to have higher numbers of CRISPRs as compared to temperate environments. This negative correlation between temperature and number of CRISPRs could be an indication of an increased viral diversity and dynamic interactions between viruses and their hosts in cold environments. In addition, we developed a workflow using CRISPRs detected in glacial ice metagenomic data to link viruses and their hosts. Infection networks were created where viruses were connected to the microbial cells they putatively infected. Additionally, we searched for transduction events in sequencing data by looking for viral sequences containing microbial DNA. Based on these results, we identified some phages that could presumably be transducing agents in glacial ice. Altogether, these results point to cold environments and specifically glacial ice as having highly dynamic viral-host interactions that can lead to transduction events and eventually to microbial adaptation.

Poster C-22

EFFECT OF PROLONGED DARKNESS AND TEMPERATURE ON THE LIPID METABOLISM IN THE BENTHIC DIATOM *Navicula perminuta* FROM THE ARCTIC

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The Arctic regions are an extreme habitat for phototrophic algae due to long periods of darkness caused by the polar night. Benthic diatoms, which dominate microphytobenthic communities, are known to survive this dark period, but the underlying physiological and biochemical mechanisms are still poorly understood. One of the potential adaptive mechanisms for long term dark survival is the utilisation of stored energy products in combination with a reduced metabolism. The main storage compound in diatoms consist of the neutral lipid triacylglycerol (TAG) which is stored in cytoplasmic droplets. In recent years water temperatures in the Arctic increased due to an ongoing warming. This could increase the cells energy requirement for the maintenance metabolism during darkness and therefore accelerate the consumption of lipid reserves. In this study we investigated the lipid metabolism of *Navicula perminuta* Grunow, an Arctic benthic diatom isolated from the microphytobenthos of Kongsfjorden (Svalbard, Norway), over a dark period of eight weeks at two different temperatures (0 and 7°C) using Fourier transform infrared (FTIR) spectroscopy, HPLC and GC-MS. The results show a decrease in the lipid to protein ratio (FTIR) over the dark period of eight weeks. HPLC separation of the lipid classes revealed a gradual reduction in the neutral lipid classes TAG and FFA (free fatty acids), and an unchanged content of the polar lipids (membrane lipids like phospho- and glycolipids) during dark treatment. Differences in temperature were conspicuous, with lower TAG content at 7°C after 8 weeks. The amount of total fatty acids (FA_{Total}) also decreased over time (GC-MS), but with differences in the relative composition of SFA, MUFA and PUFA (saturated-, monounsaturated- and polyunsaturated FA) between 0 and 7°C. The results demonstrate that *N. perminuta* relies on stored lipid reserves during prolonged dark periods, most probably to maintain a basic metabolism. Under enhanced temperatures the lipid resources were used faster, which could lead to a depletion of the energy reserves before the end of the polar night. Although the consequences are not clear at present, they might be harmful to Arctic diatom species.

KEYWORDS: DIATOMS, DARK SURVIVAL, LIPIDS, ARCTIC, MICROPHYTOBENTHOS

Poster C-23

MICROBIAL TRANSCRIPTOMIC RESPONSE TO THAWING AND FREEZING OF ACTIVE LAYER PERMAFROST SOIL

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Permafrost affected soil systems represent 17% of the global land area. Due to increasing global temperature the thickness of the above-lying active layer has increased over the past decades. The active layer of soil overlaying permafrost in the Arctic is subjected to dramatic annual changes in temperature and soil chemistry, which we hypothesize to affect microbial activity. To be able to survive these conditions microbes have different survival mechanisms, such as lipid modification to maintain cell membrane fluidity, antifreeze proteins to inhibit formation of ice crystals, and cold adapted enzymes with high specific activity at low temperatures. Most of these mechanisms have been identified and verified in pure cultures of microbes isolated from cold environments but very little is known about the mechanisms employed by soil microbial communities. Thus, we investigated the microbial responses upon thawing and again during freezing of active layer permafrost from Svalbard.
Microbial responses were investigated by incubating active layer soil at 4 °C intervals from -10 to 2 °C over 6 days. Three weeks at 2 °C were followed by freezing at 4 °C intervals to -10 °C over 6 days. Metatranscriptomic data were obtained by extracting RNA from 2 gram of soil sample, cDNA and library production using NebNext Ultra Directional RNA library prep kit, and sequencing with Hiseq 150 bp paired-end technology. Sequences were Blasted against custom-made protein data base with protein of interest in our system and NCBI nr database, using DIAMOND.
Here we present our preliminary result, showing the dominant responses of active layer soil microorganisms upon thawing and freezing at different temperatures.

KEYWORDS: METATRANSCRIPTOME, PERMAFROST SOIL, STREES DEFENSE MECHANISMS, THAWING AND FREEZING.

Poster C-24

ANTIFREEZE PROTEIN ACTIVITY IN GLACIER CRYOCONITES

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Antifreeze proteins (AFPs) are a diverse group of icebinding proteins that inhibit the growth of ice in two different situations. Prior to freezing they possess thermal hysteresis (TH) activity, which is the non-colligative depression of the freezing point of a solution containing ice below its melting point. In the frozen state they show ice recrystallization inhibition (RI), whereby the proteins inhibit the growth of large crystals at the expense of small crystals at high subzero temperatures.

'Cryoconite holes' are organically rich, variously shaped, water filled depressions is a unique habitat distributed over the glaciers. Despite the environmental stresses such as sub-zero temperatures in winter, freeze–thaw cycles, nutrient deficiency and long phase of covered by thick ice, microbial communities survive in Cryoconite holes. The bacterial strains belonging to four species, (*Pseudomonas ficuserectae, Cryobacterium psychrotolerans, Cryobacterium psychrophilum, Leifsonia* sp.) and yeast strains (*Rhodotorula* sp.,) isolated from the cryoconite hole sediments of Arctic glaciers, were subjected to screening for antifreeze proteins (AFPs). *Pseudomonas ficuserectae* exhibited a high thermal hysteresis (TH) activity at up to 2ºC. Ice recrystallization inhibition (IRI) activity was observed in most cultures at low protein concentration (0.05mg/ml). The six bacterial AFPs produced rounded shape of ice crystals that did not change their size and morphology within the TH window. SDS- PAGE analysis of the AFPs suggests their apparent molecular weights to be around 23 kDa. The Cry-C AFP from the strain *Cryobacterium psychrotolerans* was ultrafiltrated using Amicon (Millipore) with membrane cut-off of 3kDa, to remove salts and placed in ammonium acetate 25 mM as final solvent to obtain final protein concentration around 40 µM. The sample was analyzed by ESI-Q-ToF-MS at a protein concentration of 10 µM, 30% acetonitrile, 0.5% formic Acid (final) in ammonium acetate 25mM. Peak Intensity is expressed under the mass to charge ratio (m/z) value on spectra. ESI-Q TOF analysis of the Cry-C protein revealed its exact molecular mass to be 22.14 Kda corroborating its previous observation on SDS-PAGE analysis that was 23 kDa (approximately) [Lane B, Fig. E] and 21 peptides could be sequenced with a high-level confidence after multi-enzymatic digestion (MELD) and nanoLC-ESI-MS/MS analysis.

This study is significant as it screens several species of Arctic bacterial strains for AFP activity. So far only one species of bacteria, *Pseudomonas putida,* was reported from the Arctic to produce AFPs. N-terminal amino acid sequence analysis shows that the bacterial AFPs isolated belong to the AFP family IBP-1, which is known to have an important physiological role in the cold environment. The habitat from which the AFP was isolated is interesting and has been discussed.

KEYWORDS: ANTIFREEZE PROTEIN; THERMAL HYSTERESIS; RECRYSTALLIZATION INHIBITION; CRYOCONITE

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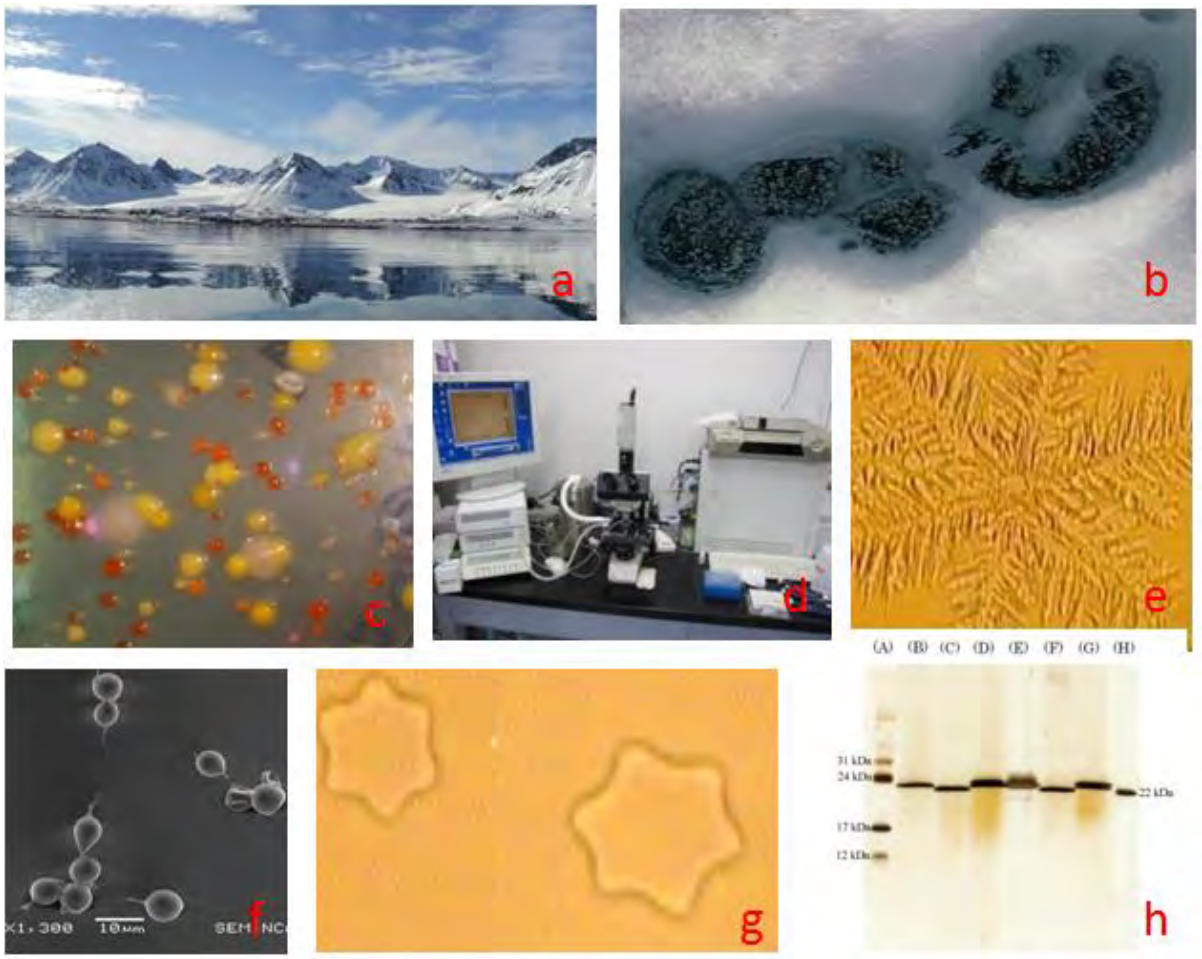

Fig. 1. a) Landscape of Svalbard glaciers, b) Cryoconites holes, c) Bacterial colonies grown on culture media, d) Photosystem consisting of a microscope and temperature controller, e) Ice growth patterns in the presence of bacterial AFPs, f) SEM of a novel yeast species (*Rhodotorula* svalbardensis sp.nov.), g) Hexagonal Ice crystal growth patterns in the presence of yeast AFPs, h) SDS-PAGE analysis following AFP purification.

Table 1. N-terminal amino acid sequences of AFPs studied. 'Tis' indicates *Typhula ishikariensis* and first 20 residues of an AFP.

Species	**Culture accession number**	**N-terminal amino acid sequences of AFP**
Pseudomonas ficuserectae (AB021378) by 96.9%	JCM 19509	----NSNPSPVXLGSAXTFAILSQ-
Pseudomonas ficuserectae (AB021378) by 97.3%		----NSNPSPVYLGSAXTFAILSQ-
Cryobacterium psychrophilum (AM410676) by 95.7%	JCM 19505	---DVMPQAPVNLGSTEXFSILS--
Cryobacterium psychrotolerans (JN637331) 98.2%	JCM 19503	AVPXGSVXAXVXXGAATTFX-----
Cryobacterium sp. AsdMX-L1 (JX123060) by 99.0%	JCM 19507	AXPGGXRXXXVXXGAATXFI-----
Cryobacterium psychrotolerans (JN637331) 99%	JCM 19506	AVPVGSVRAPVALGLAXXFA-----
Tis		-----AGPSAVPLGTAGNYVILAST

Poster C-25

COLD-ACTIVE ANTIMICROBIAL AGENTS PRODUCED BY ANTARCTIC PSEUDOMONADS

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INTRODUCTION

Antibiotic resistance is a serious problem of modern healthcare leading to intensification of new antimicrobials searching. Bacteriocins are widespead group of antibacterial proteins produced by both grampositive and gramnegative bacteria. They target only same or closely related species. Particular bacteriocin were named according to their producer, agents produced by *Pseudomonas* sp. are called pyocins. There are three types of pyocins, low molecular weight S pyocin and high molecular weight R and F pyocins. To this date, attention has been focused mainly on mesophilic bacteria, far less is known about antagonistic relations between cold-adapted microorganisms. But psychro-philic/tolerant bacteria are promising source of new bioactive metabolites because their extreme life style is connected with extraordinary properties of their metabolites, e.g. activity at low temperatures. Pyocins produced by Antarctic pseudomonads may be useful in clinical, agriculture or industrial applications.

MATERIAL AND METHODS

A total of 36 pseudomonads were isolated in James Ross Island, Antarctica in 2007–9. A double-layer plate assay was used for detection of pyocin production. Effect of medium and incubation temperature was tested using trypton-yeast agar, agar with mitomycin C and nutrient agar at temperatures 25°C and 4°C, respectively. The collection was screened in an all-by-all assay, i.e. each strain was used as a potential producer and indicator. Pyocin production was scored as S or R/F-type based on inhibition zone character. Phage tail-like particles were observed by electron microscopy using negative contrast method (FEI Morgani 280 microscope). Proteinaceous nature of S pyocins was confirmed by susceptibility to proteinase K and trypsin treatment. PCR screening was used for detection of known S pyocins. Activity of all detected pyocins was tested against collection of 111 clinical strains of *Pseudomonas aeruginosa* using the double-layer plate assay at 4°C on medium with mitomycin C.

RESULTS

Twenty-one strains (58%) were growth-inhibitor producers. All suspected pyocins were confirmed by susceptibility to proteases and electron microscopy, respectively. Nine strains produced S pyocin, whereas15 strains were producers of R pyocin. No F pyocin producer was detected. Three strains secreted S and R pyocin together. The incubation temperature significantly influenced pyocin production (two tailed Fisher's exact test; $p = 0.002$) with higher production at4°C. Influence of medium was not statistically significant. PCR detection of known pyocins was negative in all cases which implied we worked with new types of pyocins. *Pseudomonas prosekii* 2406 produced R pyocin killing 46.8% of clinical strains, whereas *Pseudomonas* sp. 2663 produced S pyocin with activity against 9.0% of strains.

DISCUSSION

Pyocin production of Antarctic pseudomonads is lower compared to mesophilic strains (Michel-Briand et Baysse 2002). Low cultivation temperature boosted pyocin production which corresponded with previous findings that some cold-adapted bacteria produce inhibition agents only at low temperature (Sánchez et al. 2010). We assumed that low temperature is kind of stress and bacteria coped with it by pyocin production. Most likely, we detected the new types of pyocins, but this will be confirmed by hybridization assays. The bacteriocins of non-pathogenic species could inhibit growth of clinical important strains, like in case of colicin Fy (Bosák et al. 2012). Also two Antarctic environmental strains inhibit clinical isolates of *P. aeruginosa*. Whereas strain 2663 produced pyocin only at 4°C, pyocin of strain 2406 was produced both at 4°C and 25°C, but with higher production was obtained using lower temperature. Both pyocins are stable at 37°C because this temperature was used for cultivation after overlay with indicator strain.

In conclusion, Antarctic soils showed to be reservoir of novel cold active antimicrobials with possible impact on clinical important strains.

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KEYWORDS: COLD-ACTIVE METABOLITES, ANTIMICROBIALS, *Pseudomonas*, BACTERIOCIN, ANTARCTICA

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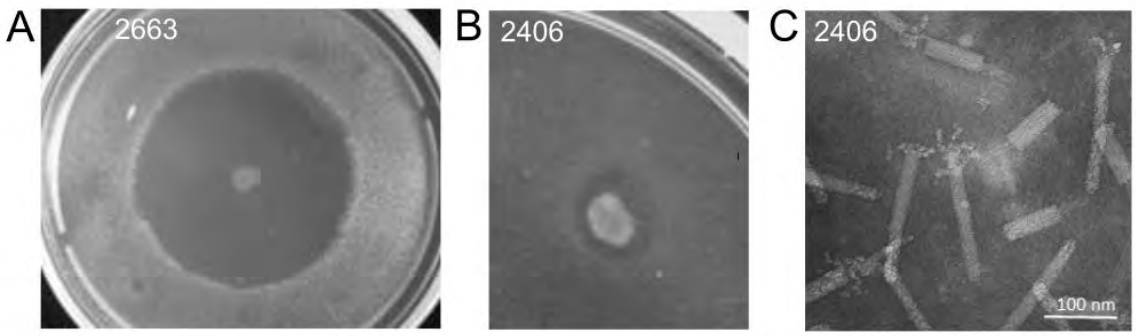

Fig.1. Pyocins.

Poster C-26

PHYLOGENETIC, STRUCTURAL AND NUCLEIC ACID BINDING PROPERTIES OF A NOVEL TYPE OF RNA-BINDING (TRAM) PROTEIN FROM AN ANTARCTIC ARCHAEON.

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Proteins with a single TRAM domain appear to be unique to Archaea. Despite the presence of these genes in many members of this domain of life, their function(s) has not been experimentally determined. Based on our experimental findings and the crystal structure of one protein from Methanosarcina mazei, TRAM proteins are predicted to bind RNA. The protein has a fold with anti-parallel beta sheets, similar to cold shock domain (CSD) proteins which are absent from most Archaea. In the Antarctic methanogen, *Methanococcoides burtonii*, three TRAM domain proteins (Mbur_0304, 0604 and 1445) were determined by proteomics to be some of the most up-regulated proteins during growth at low temperature. Due to their specific inferred role in cold adaptation, the lack of typical CSD proteins in Archaea (which can play a role in cold adaptation in Bacteria), and the general lack of experimental data defining the function of this class of proteins, our research is invaluable in regards to characterizing the biophysical and nucleic acid binding properties of one *M. burtonii* TRAM domain protein (Mbur_1445) and mapping protein-nucleic acid interaction networks. Our study has determined evolutionary and structural properties of TRAM proteins in M. burtonii, revealing unique characteristics related to RNA binding. The sequencing of bound RNA has provided great scope and opportunity for learning about the biological function, including its role in cold adaptation of *M. burtonii* and relevance of this novel class of nucleic acid binding protein, TRAM Mbur_1445, and its broader function in Archaea and possibly Bacteria.

Poster C-27

CRYSTAL STRUCTURE AND EXPRESSION OF A PUTATIVE PHAGE-LIKE PROTEIN CODED IN THE GENOME OF A MARINE ANTARCTIC BACTERIA

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INTRODUCTION

Bizionia argentinensis JUB59^T (*B.a*) was isolated from surface marine water in Potter Cove, (62° 149´ S; 58° 409´ W), Antarctica and described as a new species. JUB59 is a psychrotolerant marine bacteria; Gram-negative, non-motile rods forming yellowish orange colonies on marine agar (Bercovich et al. 2008). The sequencing of JUB59^T genome (GenBank AFXZ00000000) suggested interesting features such as UV resistance, hydrolytic exoenzymes, and nitrogen metabolism. Several putative gene sequences, of unknown biological function and with possible new folds were selected, cloned, expresed and its products were purified from the culture broth. One of the *B.a* gene products, named C24, showed structural homology with a T4 phage tail fiber protein. In this work, we investigate the structure and expresion of C24.

METHODOLOGY

C24 gene was amplified by PCR from *B.a* chromosomal DNA, cloned into the expression plasmid pDEST-527 and expressed in *E. coli* BL21(DE3). The protein was purified using a HisTrap HP column, dialyzed and concentrated. Rhomboidal-shaped crystals with a maximum size of 0.3 mm x 0.2 mm x 0.1 mm were obtained and analyzed at 100 K at the PROXIMA 1 protein crystallography beamline at the SOLEIL synchrotron, France. Superpositions and rmsd calculations were done with the PDBeFold server and the study of interfaces, monomers and assemblies with the PDBePISA server at the EBI. The likely induction by stress of C24 expression in *B.a* was tested in Erlenmeyer flasks with 30 ml of marine broth (10°C and 200 rpm). When cultures reached stationary phase, one was left as a control and the others were subjected to different treatments [shift to high (28 °C) and low (0 °C) temperature, high (9 g l^{-1}) and low (0.8 g l^{-1}) salinity, oligotrophy and presence of other related (*B. myxarmorum* ADA-4^T) and non-related (*Cellulophaga* sp. S03-8, *Pseudomonas* sp. P96-50 and *Shewanella* sp. Ag06-30) Antarctic bacteria]. Samples were taken after 1h and 7h, total RNA was extracted and the presence of C24 transcripts was detected by PCR using specific primers and the products obtained were sequenced to confirm their identity. The same primers were used to detect the presence of C24 gene in other Antarctic bacterial isolates.

RESULTS

A construct harboring the bzarg_797 gene from *B.a* was expressed and the structure of the 277-residue protein called C24 was solved. A unique 89.1 kDa trimer was found in the asymmetric unit of the crystal, which corresponds to the expected biological assembly of the protein. The protein binds a unique Mn (II) ion which is octahedrally coordinated by six histidine residues. C24 bears four distinct domains, with contribution of the three chains in each of them: i) a globular, unique proximal shoulder domain, ii) a globular collar domain, iii) an elongated, intertwined metal-binding needle domain, and iv) a globular, receptor-binding-like unique distal head domain. The structure of C24 partially resembles that of the receptor-binding tip from the bacteriophage T4 long tail fiber, yet there are marked differences between both in their domain organization, size, sequence identity and metal binding nature. Both structures present a similar overall shape, but they can be superimposed only in the collar, intertwined and needle regions, with the metal binding sites being different in number and also slightly shifted between proteins. The structure similarity search on the globular proximal shoulder and distal head domains returned no homologue partners. The bacteriophage T4 long tail fiber head domain, despite showing an analogous threaded architecture, fails to superimpose both in structure and in primary sequence to C24. In the latter viral domain, exposed aromatic and positively charged residues are proposed to interact with sugar residues and their attached phosphate groups, respectively, in *E. coli* host cell receptors. Remarkably, the head domain from C24 also presents several positive and aromatic exposed residues with unknown function so far.

The C24 gene was not detected in other *Bizionia* type strains nor in 18 Antarctic isolates of different genus, among which 11 were obtained from Potter Cove. After growing *B.a* at 10 °C, C24 was expressed and could be detected by RT-PCR in the control culture and in all of the other stress-induced conditions tested, suggesting a constitutive expression of C24 gene at least under the culture conditions used.

DISCUSSION

C24 expression seems not to be related with response to stress induced by sudden shifts to extreme environmental change or to the presence of other possible competitor bacteria. It seems to be constitutively expressed at least at the culture conditions used. At present, the role of C24 in *B.a* could not be elucidated. One possibility is that C24 be a relict from a former lysogenic phage, as at least three different prophages were detected in *B.a* genome, that were excised after mitomicin C treatment. It could also be related to a type VI transport system or be involved in 'social' behavior like biofilm fomation or attachment to surfaces or even to the growth in other environmental situations non tested so far. Further studies will be conducted with the aim of knowing the role C24 expression has in *B.a* strain and its likely presence in other bacteria.

KEYWORDS: PROTEIN STRUCTURE, ANTARCTIC BACTERIA, X-RAY CRYSTALLOGRAPHY, *Bizionia*

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Session D
Supraglacial, subglacial and glacial microbiology

Keynote lecture KN-D

THE ECOLOGY AND BIOGEOCHEMISTRY OF MARITIME ANTARCTIC SNOW

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Our understanding of the ecology of Antarctica's largest terrestrial habitat, ice, is arguably least developed where it is most active: within seasonally melting coastal snowpacks. Melting snow contributes about 350 km^3 yr^{-1}, or ca. 83%, of all surface and near-surface melting in Antarctica, and covers up to 20% of its surface area according to distributed surface energy balance models (Liston et Winther 2005). According to Abram et al. (2013), this melting is now occurring at an unprecedented rate relative to the last 1000 years in the Antarctic Peninsula region. However, there are so few insights into how biogeochemical and biological processes are enhanced by this melting that we have little idea of its impacts. The paucity of ecological studies of snowpack habitats must therefore be addressed so that we can quantify changes in *in-situ* microbial processes and address the enormous uncertainty that exists with respect to how the export of labile nutrients and viable microorganisms by runoff might influence downstream terrestrial and marine ecosystems.

Snowpack ecosystem studies in coastal Antarctica, especially in the Antarctic Peninsula region, are therefore urgently required, and this talk will demonstrate some of the key challenges they face. In so doing, recent experiences mapping biomass distribution using optical techniques, measuring biological production from radiolabel incorporation and quantifying net ecosystem exchange (NEE) from measurements of CO_2 will be described. New insights into the ecology of snowpacks in the South Orkney Islands and the South Shetland Islands will also be presented. These include the development of extreme chemical conditions at the base of the snowpack (where many microorganisms are initially present during the summer) and harsh abiotic conditions that can prevent the surface manifestation of snow algae and other photoautotrophs due to burial and impose water supply restrictions due to re-freezing. It will be demonstrated that these greatly influence NEE measurements and, along with photochemical transformation of organic carbon, mean that the estimation of biological production from snowpack and/or boundary layer CO_2 profiles is extremely difficult to achieve. It will, however, be shown that the depletion of CO_2 in snowpack air by autotrophic activity can be detected during stable weather conditions.

The above case studies also reveal significant heterogeneity within maritime Antarctic snowpack ecosystems. For example, horizontal, metre-scale patchiness of snowpack microbiota characterised two snowfields influenced by allochthonous nutrient inputs from marine fauna and dust in the South Orkney Islands. In addition, there was a marked difference in the microbial biomass and community composition of coastal snow patches compared with that found on nearby (and more expansive) glacial snowpacks in the South Shetland Islands. The effects of marine fauna were also important here, producing intense surface algal blooms in the coastal snow patches that resulted in a drastic reduction of snow surface reflectance, and therefore enhanced the rate of meltwater production by darkening the snow. However, when these blooms were buried by fresh snow, the rate of melting dropped markedly, the CO_2 content of the snow increased, and the autotrophic community was undetectable from surface reflectance properties. Instead, we found that spectral transmission profiles through the snow provided a quantitative basis for estimating the underlying autotrophic biomass. The optical, chemical and physical properties of maritime Antarctic snow are therefore intricately linked to its resident microbial ecosystem.

KEYWORDS: SNOW ECOLOGY, SNOW ALGAE, OPTICAL BIOGEOCHEMISTRY, ANTARCTICA

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Lecture D-01

LIGHT ADAPTATION OF MICROBIAL COMMUNITIES IN ANTARCTIC CRYOCONITE HOLES

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Cryoconite holes and cryolakes are important refugia for microorganisms on the surface of glaciers worldwide. Those in the McMurdo Dry Valleys are unique in being covered by an ice lid for the majority of the ablation season. They contain concentrations of photosynthetic organisms, including cyanobacteria and some green algae. These communities recycle nutrients and can generate significant quantities of bioavailable carbon. We conducted temperature and light controlled laboratory incubations to determine how physical conditions constrain the efficiency of the photosynthetic community. The incubations were conducted over long time scales, from weeks to months, to simulate conditions in the field. By using oximetry and variable chlorophyll fluoresence, we found that the communities are well-adapted to low light levels and become less efficient when exposed to high light. This demonstrates that the ice lid has a marked influence on the cryoconite community, and reveals an important difference between Arctic supraglacial communities, which are generally exposed to high light levels throughout the ablation season, and Antarctic cryoconite holes and supraglacial lakes which retain an ice lid. This has implications for estimates of carbon production by supraglacial communities, and on the relative contribution of cryoconite holes to total carbon export from glaciers and ice sheets.

KEYWORDS: CRYOCONITE, SUPRAGLACIAL, CARBON, PHOTOSYNTHESIS, ACCLIMATION

Lecture D-02

EXPORT OF MICROBIAL CELLS FROM THE GREENLAND ICE SHEET

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The bed of the Greenland Ice Sheet (GrIS) contains an active microbial community. The end products of its metabolisms are CO_2 and, due to the mostly anoxic conditions, also CH_4, both climate amplifiers. The ice sheet has been undergoing an accelerating net mass loss in response to warming air temperatures, with meltwater runoff responsible for more than a half of the total mass loss. Fluvial transport is the major mechanism exporting substantial amounts of subglacial sediment towards the ocean, and is frequently dominated by extreme events. We hypothesised that subglacial methanogens (CH_4 producers) are transported along with subglacial sediment and may resume their activity when conditions become favourable again, and that significant amounts of CH_4 can be produced in these proglacial environments as a direct effect of export of subglacial methanogens. To test this hypothesis, we investigated the abundance and diversity of microbial cells in a river draining a large catchment of the southwestern part of the GrIS in the record melt season 2012, using discharge and sediment loading data from hydrological measurements, and quantitative PCR analysis and Illumina sequencing of DNA extracted from the river sediment. We also collected freshly deposited sediment from the river delta and used it for long-term laboratory incubation experiments (six weeks), during which we measured CH_4 production and monitored the concurrent changes in chemistry and microbial diversity. We show that significant numbers of viable microbial cells are exported from beneath the ice sheet, providing organic substrate for downstream ecosystems and potentially contributing to the production of climate amplifiers in these systems.

KEYWORDS: GREENLAND ICE SHEET, SUBGLACIAL SEDIMENT, GLACIAL EXPORT, MICROBIAL ACTIVITY, METHANOGENESIS

Lecture D-03

DYNAMICS AND MICROBIAL COMMUNITY FUNCTIONS IN CRYOCONITE FROM ITALIAN ALPS AND KARAKORAM

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INTRODUCTION

Most of the studies on the ecology of cryoconite holes focused on Arctic and Antarctic ice sheets while mountain glaciers remain under investigated. Indeed, to the best of our knowledge, the only studies dealing with the ecology of cryoconite holes outside polar areas are those by Edwards and co-workers (Edwards et al. 2013, Edwards et al. 2014) and Segawa and co-workers (Segawa et al. 2011). In this study we aimed at contributing to filling this gap of knowledge by investigating two aspects that have been poorly studied so far. First, we investigated the temporal dynamics of the microbial communities in cryoconite holes along the ablation season. For this purpose, we collected samples of cryoconite from the bottom of cryoconite holes on the Forni glacier (Italian Alps) in July, August and September 2013. The same hole was sampled in different months whenever possible, and the microbial community structures were assessed through Illumina sequencing of a fragment of the 16S rRNA gene. Second, we aimed at assessing the ecosystem functions of cryoconite microbial communities by the means of metagenomics approach. To this end, we collected also cryoconite samples from Baltoro glacier (Karakoram) and we applied Whole Metagenomic Sequencing (WMS) of the DNA extracted from 10 selected samples collected on Forni and Baltoro glaciers.

METHODS AND MATERIALS

During the ablation season (mid-June-September) 2013 we collected samples from 45 different cryoconite holes on Forni Glacier (Italy) during three visits conducted on July 10th, August 28th, and September 25th. Samples from 30 different cryoconite holes on Baltoro glacier were collected from 30th June to 1st July 2013. Respiration rate and net community production were estimated by ΔO_2 method. Organic matter content of cryoconite was measured with loss-on-ignition method. On all the samples the V5-V6 hypervariable regions of the 16S rRNA gene were PCR-amplified and sequenced by MiSeq Illumina (Illumina, Inc., San Diego, CA, USA) using a 250 bp x 2 paired-end protocol. WGS was performed on 5 samples from Baltoro and 5 from Forni by HiSeq Illumina (Illumina, Inc., San Diego, CA, USA) using a 100 bp x 2 paired-end protocol. Amplicon data were analysed on the basis of Operational Taxonomic Units (OTUs) defined at 97% of similarity. Ecological determinants of the composition and structure of microbial communities were investigated by redundancy analyses (RDA) based on Hellinger distances among communities. Metagenomic data were analysed using MG-RAST pipeline and STAMP.

RESULTS

On Forni glacier, cryoconite microbial communities were strongly dominated, in all months, by Cyanobacteria and Sphingobacteriales (17.1% and 19.8% on average, respectively), with smaller percentages of Burkholderiales, Pseudomonadales and Actinomycetales. A relevant abundance of Actinobacteria, unclassified at Order level, was also observed, especially in samples collected in July and August. Redundancy Analysis (RDA) indicated that structure of bacterial communities changed between months. Post-hoc tests also disclosed significant differences between all pairs of months (Pseudo-$F_{1,38} \geq 1.821$, $P_{FDR} \leq 0.047$ in all cases). However, the age of the hole did not predict the bacterial community structure. Structure of bacterial communities also changed according to position of cryoconite holes in the study area, as indicated by the significance of coordinates, and to the amount of organic matter in the cryoconite (Fig. 1).

Cyanobacteria represented up to 24.6% of the entire community in July but only 13.3% in both August and September. High abundance of Cyanobacteria is in contrast with respiration data, which revealed that cryoconite holes displayed a net respiration activity. Conversely, heterotrophic populations increased from July to September. Particularly, Sphingobacteriales (phylum Bacteroidetes) increased from 14.3% in July to 23.1% and 22.0% in August and September, respectively. We also investigated community similarity in space. Mantel correlograms showed that in July and September communities close to each other were more similar than distant communities (details not shown).

Preliminary results from metagenomics revealed that Forni and Baltoro cryoconite are functionally different; particularly, reads related to photosynthesis (SEED- level 1) were almost twice as abundant on Forni as on Baltoro Glacier (Fig.2).

DISCUSSION

The decrease of photosynthetic bacteria along the ablation season observed on the Forni glacier suggests that an ecological succession actually occurs on glacier surface, with autotrophic populations colonizing the cryoconite after snow melting. Subsequently, these populations reduced their abundance in favor of heterotrophic populations. We stress, however, that this ecological succession should occur consistently in the cryoconite on the glacier surface, and not within each single hole, because this study indicates that bacterial community structure is not related to the time since hole formation. That means that, e.g. in August, cryoconite holes hosted bacterial communities typical of August, regardless of whether they formed in July or August. The significant similarity of structure of bacterial communities in holes at short distance suggests that short-range transport through hydraulic connection among close cryoconite holes might have a homogenizing effect on the microbial community structures of holes. This process may contribute to explain also high similarity of bacterial communities in cryoconite holes with different ages collected in the same month.

KEYWORDS: CRYOCONITE, ALPS, KARAKORAM, METAGENOMICS, SUCCESSION

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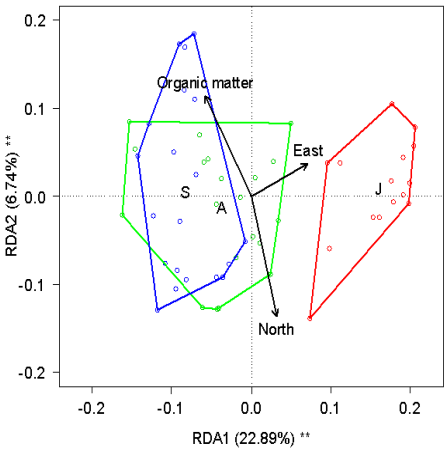

Fig. 1. Biplot of the bacterial communities in cryoconite holes on Forni glacier. Each circle represents the bacterial community in a cryoconite hole.

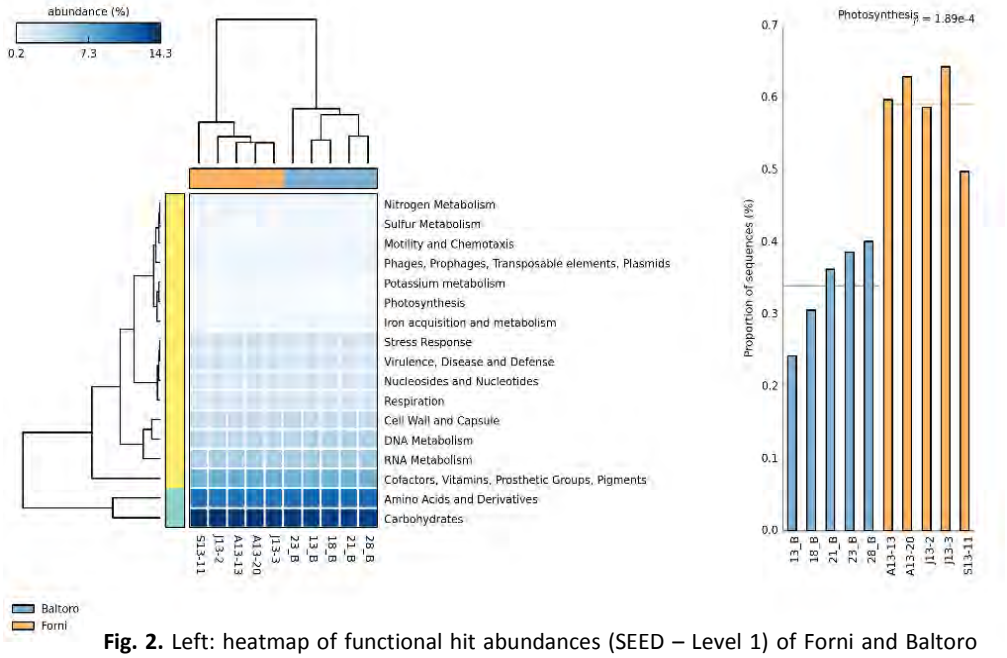

Fig. 2. Left: heatmap of functional hit abundances (SEED – Level 1) of Forni and Baltoro cryoconite. Right: bar graph of hits annotated as “photosynthesis”

Lecture D-04

BIOGEOGRAPHY AND FUNCTIONALITY OF MICROBIAL GLACIAL SURFACE COMMUNITIES ACROSS THE ARCTIC.

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Glaciers are important components of Earth's climate and water system. Physical and chemical aspects of glaciers have been studied intensively; however, glacial microbiology is still in its infancy. Glacial surfaces have been considered barren for a long time, yet distinct habitats can be found to harbour species from all three domains of life. We recently showed that snow and ice algae – polyextremophile microeukaroytes – are critical and prolific primary colonisers and producers in these environments (Lutz et al. 2014). Furthermore, due to the development of pigmentantion they have a significant effect on surface albedo. However, the relationships between environmental conditions and microbial abundance, diversity and function on snow and ice surfaces in the Arctic are still poorly understood.

Here we present the first comprehensive metagenomic study of various snow and ice habitats from 21 glaciers spanning the European Arctic (Svalbard, Sweden, Iceland, Greenland) sampled during the 2012-2014 melting seasons. The biome composition and function and the geochemical parameters that affect their growth have been characterized. Our results reveal a cosmopolitan distribution of snow and ice algae and that our habitat classification is valid across all studied Arctic glaciers. Algal composition within a single habitat is more similar than within a geographic location. On the other hand, the bacterial composition shows a more endemic distribution. Archaea were also detected in all samples. Overall functionality (e.g., metabolic fingerprints, pigments, fatty acids) seems to be controlled by nutrient availability and trends for C/N/P ratios could be established.

KEYWORDS: SNOW AND ICE ALGAE, BACTERIA, ARCHAEA, C/N/P RATIOS, METABOLITES

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Lecture D-05

MICROBIAL LIFE IN THE ARCTIC SNOWPACK PHOTOCHEMICAL REACTOR

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Winter snow can cover up to 12% of the Earth's surface, which is about 61 millions of square kilometer. This porous media with high surface area, light transmittance and permeability has been described as a photochemical reactor, which strongly interacts with the overlying atmospheric layer. Given its massive coverage and chemical reactivity, the snowpack could have a major role in (bio)geochemical cycling at a global scale. However, the potential biological participation within this system remains largely unknown. Numerous studies have described the occurrence and diversity microorganisms in snowpacks. Here, we investigated snow microbial inhabitants and ecology by addressing i) how a microbial community might be selected after atmospheric wet and dry deposition and ii) what mechanisms might drive microbial colonization within the highly photochemically active snow matrix. We compared arctic spring snow and atmosphere for their taxonomical composition and functional abundance and diversity using a metagenomic approach. Microbial community composition and function strongly differed between atmosphere and surface snow and the dissimilarity increased with time after atmospheric seeding via a deposition event. Functions related to UV-screening compound synthesis, oxidative stress response and DNA repair mechanisms have been detected in snow metagenomes with high relative abundance, which decreased with depth. Snowpack microbes might develop a wide range of strategies to inhabit snow and might form an active microbial community with an underestimated role in global biogeochemical processes.

Lecture D-06

SETTLEMENT OF AN ALPINE ENGLACIAL SYSTEM WITH MICROBIAL COMMUNITIES – WHO COMES FIRST?

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Supraglacial systems harbour active microbial communities and are subject to numerous metabolic processes which in ablation periods peak in high productivity. Contrary to that, the englacial compartment apparently lacks this diversity and activity - with the exception when a connectivity with the atmosphere exists as well as access to humans (Mulec 2014).

In this case we investigated a glacier cave „Hintertuxer Natur Eis Palast“, located at 3.250m a.s.l. in the Austrian Alps. It is a natural crevasse under ca. 30m of ice with ultra-oligotrophic conditions and high humidity close to 100%. It is accessible and also open to the public as a touristic highlight. In order to lead often untrained people safely through the cave the terrain is illuminated with light sources of different wave lengths. Those light sources result in the growth of so called „lampenflora“ which are microbial assemblages consisting of bacteria, algae, fungi and also protozoa. Depending on the wave length and the distance of the light source they show extremely high productivity (Larch 2014). Darker areas reveal relatively slow growth of bacteria and algae as well as low productivity. Eventually the operator of the cave connects newly discovered chambers with artifical tunnels which are then subject to new settlement. Beside the investigation of natural sources such as melt water and air currents artificial origins and processes for this inoculation are subject of this study.

To investigate the sequence of microbial settlement of freshly exposed ice we therefore sterilized the ice by melting it with subsequent filtration by 0,22µm and refreezing it in sterile containers. Duplicate boxes have been placed in a chamber which was inaccessible to tourists and have been exposed under dark conditions and different light sources. Those containers have been sampled regularly over a period of 16 weeks for bacterial cell numbers, primary and bacterial production as well as microbial biodiversity (MySeq Illumina). In order to assess the impact of tourists (of which the operator counts ca. 100.000 per year) we investigated shallow ice cores of those ice walls which are regularly touched by humans for diversity (MySeq Illumina). To estimate the microbial load they potentially introduce into the cave the shoes have been sampled outside before and after visiting the system by specific contact plates and evaluated via Laser-Desorption/Ionisation Time of Flight mas spectrometry (MALDI-TOF). Additionally, air samples have been taken with 2 types of airsamplers (Sartorius MD8 and Coriolis µ).

Results revealed a surprisingly fast settlement within 2 weeks with air streams as the main inoculation source of airborne spores of algae which inoculate the ice surface. Subsequently, cyanobacteria may settle depending on wave lengths of the light sources. According to our 16-week study, protozoa are the last in the assemblages, hypothetically entering the system with melt water through the ice matrix. In contrast to the illuminated boxes those containers which have been exposed in the dark showed extremely slow settlement with very low biodiversity. Tourists, however, leave a massive footprint in this pristine system by introducing a variety of pathogens and microbial assemblages which are well known from the human skin biome. If they are viable in these conditions is still questionable.

Accessability of this crevasse to this high amount of tourists is altering the microbial settlement substantially by usage of artificial illumination and also by active introduction of cells by shows and skin contact. Hence, large areas can be altered into highly active ecosystems with partly non-indigenous microbial assemblages.

KEYWORDS: ENGLACIAL SYSTEM, LAMPENFLORA, INOCULATION SOURCE, AIRBORNE, TOURIST IMPACT

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Lecture D-07

THE NITROGEN CYCLE IN CRYOCONITES: NATURALLY OCCURRING NITRIFICATION-DENITRIFICATION GRANULES ON A GLACIER

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Microbes occasionally form granular structures in both natural and artificial environments. Cryoconite granules are naturally occurring spherical structures, with a diameter ranging from 0.2–2 mm, which are frequently found on the surface of glaciers. Cryoconites have a significant influence of glacier mass balance because the granules decrease surface albedo, ratio of reflected flux to incoming flux of solar radiation, and accelerate the melting of snow and ice. While it has been postulated that the microbes in cryoconite granules play an important role in glacier ecosystems, information on their community structure is still limited and their functions remain unclear.
In the present study, we collected cryoconite granules from a glacier in Central Asia, the Urumqi Glacier No.1 in China. Ecosystems on Asian glaciers have characteristics distinct from those on polar glaciers. There are higher levels of chemical solutes in the snow on Asian glaciers than on polar glaciers, a result of the deposition of airborne desert dust and anthropogenic pollutants. Consequently, there is a greater microbial biomass and there are more abundant cryoconite granules on Asian glaciers compared with polar glaciers, resulting in a greater reduction in the surface albedo. Nevertheless, microbial nitrogen cycling on glaciers has been studied almost exclusively in polar regions, and data from Asia are scarce.
Here, we present evidence for the occurrence of nitrogen cycling in cryoconite granules on a glacier in Central Asia (Fig. 1). We detected marker genes for nitrogen fixation, nitrification, and denitrification in cryoconite granules by digital PCR, while digital RT-PCR analysis revealed that only marker genes for nitrification and denitrification were abundantly transcribed. Analysis of isotope ratios also indicated the occurrence of nitrification; nitrate in the meltwater on the glacier surface was of biological origin, while nitrate in the snow was of atmospheric origin. The predominant nitrifiers on this glacier belonged to the order Nitrosomonadales, as suggested by amoA sequences and 16S rRNA pyrosequencing analysis. Our results suggest that the intense carbon and nitrogen cycles by nitrifiers, denitrifiers, and cyanobacteria support abundant and active microbes on the Asian glacier.

KEYWORDS: GLACIER, CRYOCONITE, NITROGEN CYCLE, BACTERIA, RNA

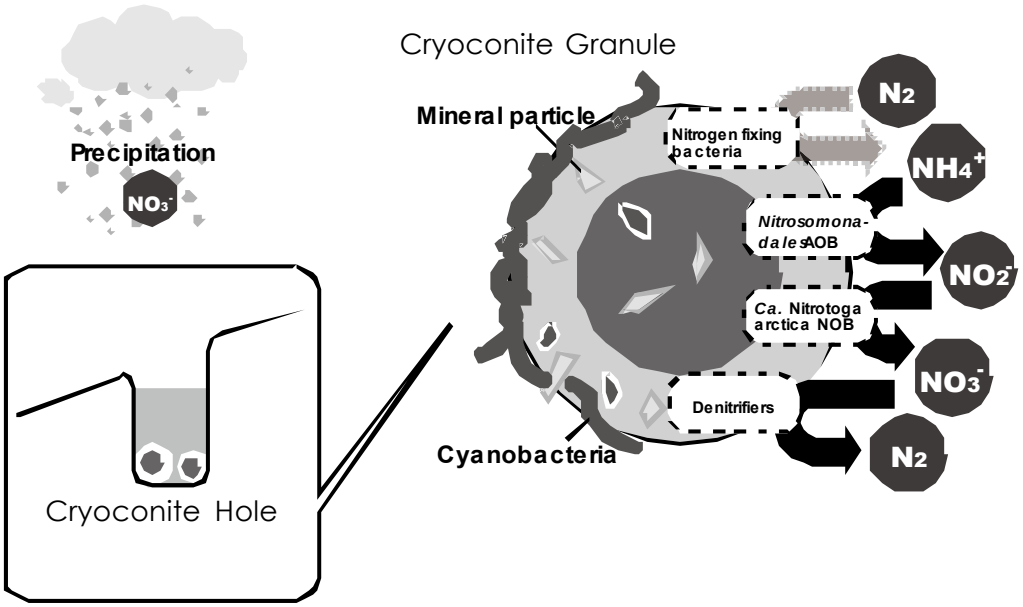

Fig. 1. Schematic diagram of the nitrogen cycle in supraglacial cryoconite granules.

Lecture D-08

LINKING ELEMENTAL CYCLES IN SUBGLACIAL SYSTEMS THROUGH MICROBIAL PROCESSES

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Glacial communition of bedrock generates fresh mineral surfaces capable of sustaining microbial communities under the dark conditions that pervade subglacial habitats. Aqueous geochemical data indicates the aerobic oxidation of FeS_2 in subglacial sediments is an important contributor to total subglacial solute fluxes via the production of protons capable of driving mineral weathering and it has been argued that this process is microbially mediated (Sharp et al. 2002, Tranter et al. 1997, Tranter et al. 2002). This inference has been supported by molecular data which has shown the presence of a number of taxa in subglacial systems closely related to known organisms capable of Fe and S oxidation (Boyd et al. 2014, Christner et al. 2014, Hamilton et al. 2013, Lanoil et al. 2009, Mitchell et al. 2013, Skidmore et al. 2005). Geochemical data has also been used to infer anoxic FeS_2 oxidation but no known mechanism exists for this process at the circumneutral pH characteristic of many subglacial waters. Therefore, questions regarding oxic, hypoxic or anoxic FeS_2 oxidation in the subglacial system remain and the role of microbes in these geochemical transformations is enigmatic. We designed and executed experiments to address these questions using subglacial sediments from Robertson Glacier (RG), Canadian Rockies and an investigative approach combining laboratory microcosms with molecular-based analyses of field samples. Quantification and sequencing of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) transcripts suggests that populations closely affiliated with *Sideroxydans lithoautotrophicus* and *Thiobacillus denitrificans*, iron-sulfide oxidizing autotrophic bacteria, are abundant components of the autotrophic community in RG sediments (Boyd et al. 2014). Microcosm experiments also indicate sulfate production during assimilation of radiolabeled bicarbonate at low temperatures (4°C). Collectively, these data suggest a role for biological pyrite oxidation in driving primary productivity and mineral dissolution in the RG subglacial environment (Boyd et al. 2014). We also have the first rate-estimate for bicarbonate assimilation in these ecosystems, demonstrating the linkage between the carbon and sulfur cycles. Reduced sulfur intermediates released during the abiotic aerobic oxidation of FeS_2 such as thiosulfate are metastable in O_2 rich waters at circumneutral to alkaline pH, characteristic of subglacial outflowing waters. We quantified aerobic and anaerobic sulfate production using thiosulfate, at low temperatures (4°C) by *Thiobacillus* sp. RG4, a chemolithoautotroph isolated from Robertson Glacier (RG) sediments (Harrold et al. in prep.). Evaluation of the *Thiobacillus* sp. RG4 genome indicates a high degree of similarity with *Thiobacillus denitrificans* and under anaerobic conditions we demonstrate the RG4 isolate coupling oxidation of thiosulfate to the reduction of nitrate or nitrite at 4°C, a temperature relevant to the subglacial environment (Harrold et al. in prep.). Further, phylogenetic reconstruction of translated *soxB* transcripts from RG sediments revealed five distinct clusters one of which included the SoxB sequence from *Thiobacillus* sp. RG4. Collectively, these data show additional mechanisms for primary production based on compounds derived from pyrite and highlight linkage between the carbon, sulfur and nitrogen cycles in the subglacial environment (Harrold et al. in prep.).

The results generated have three main implications. First, microbial activity links the carbon, sulfur and nitrogen cycles in RG subglacial sediments. Second, phylotypes closely related to *S. lithoautotrophicus* and *T. denitrificans* have been found in numerous subglacial systems suggesting that the processes identified from RG sediments are potentially widespread in subglacial systems. Third, evidence for lithotrophic primary production in this contemporary subglacial environment provides a plausible mechanism to explain how subglacial communities could be sustained in near isolation from the atmosphere during glacial-interglacial cycles.

KEYWORDS: SUBGLACIAL ENVIRONMENTS, PYRITE, PRIMARY PRODUCTIVITY

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Lecture D-09

THE ROLE OF ICE ALGAE IN THE ALBEDO FEEDBACK ON THE GREENLAND ICE SHEET

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The Greenland Ice Sheet (GrIS) has been darkening since the year 2000 due to the combination of an increasing area of bare ice, increasing near surface liquid water production, and increasing surface concentrations of light-absorbing impurities. Light-absorbing impurities found in Greenland snow and ice include mineral dust, black carbon, and microbial cells. Microbial activity is arguably the least known factor responsible for the darkening of glacier ice. It has been demonstrated that several species of algae are abundant on the ice sheet surface in Greenland. The ice algae produce a protective dark pigment when exposed to high intensities of visible and ultraviolet radiation, and intact cells absorb light with characteristic spectral profiles across ultraviolet and visible wavelengths, while inorganic dust particles display little absorption. This dark pigment not only allows the algae to survive and photosynthesise in this extreme environment, but may also decrease the albedo of the ice when present in high concentrations, potentially promoting ice melt. However, no quantitative relationship between algal abundance in the ice and albedo has been established to date. We present for the first time a quantitative estimate of radiative forcing of ice algae at the surface of the Greenland Ice Sheet. We collected data of surface albedo and ice algal abundance and biovolume over 57 days in summer 2014 in the SW part of the ice sheet, where a dark region appears every summer. This region contains dust deposited 700-2000 years ago which is now melting out and is considered the principal factor responsible for the observed albedo reduction. However, we bring evidence that algae growing in the surface ice are more significant for albedo than the outcropping dust, and argue that the effect of algae on albedo will become more important in the future.

KEYWORDS: ICE ALGAE, CELL ABUNDANCE, ALBEDO FEEDBACK, ICE MELT, GREENLAND ICE SHEET

Lecture D-10

BETWEEN A ROCK AND A HARD PLACE: ROCK COMMINUTION AS A SOURCE OF HYDROGEN FOR SUBGLACIAL ECOSYSTEMS

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Glaciers, ice sheets and ice caps presently cover ~11% of the earth's land surface. The beds of these ice masses have significant sectors at the pressure melting point, with the resulting water harbouring subglacial microbial ecosystems capable of affecting global carbon and weathering cycles, and influencing productivity in adjacent oceans through subglacial outflows at ice sheet grounding lines. It has been proposed that subglacial habitats acted as refugia to help preserve global biodiversity during past global Neoproterozoic glaciations. This has been questioned, however, since overridden photosynthetically derived organic carbon will become increasingly recalcitrant over glaciations lasting millions of years. Here we investigate the potential for abiogenic H_2 produced during rock comminution to provide a continual source of energy to support subglacial life. We crushed a range of silicate rocks to varying surface areas under an inert atmosphere, added water, and measured H_2 production with time. H_2 was produced at 0°C in all silicate rock-water experiments, likely via the reaction of water with mineral surface silica radicals formed during rock comminution. No H_2 was produced in crushed calcite control experiments. Sufficient H_2 was produced from silicate-rock reactions to support previously measured rates of methanogenesis under a Greenland glacier. We conclude that H_2 generation from glacial bedrock comminution provides a mechanism to support life and potentially help preserve biodiversity in subglacial refugia during past extended global glaciations.

KEYWORDS: SUBGLACIAL, ECOSYSTEMS, HYDROGEN, ROCK COMMINUTION, NEOPROTEROZOIC GLACIATIONS

Poster D-01

MICROBIAL COMMUNITY DYNAMICS IN THE FOREFIELD OF GLACIERS – A MODELLING PERSPECTIVE

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Glaciers and ice sheets in polar and alpine regions are retreating in response to recent climate warming, exposing terrestrial ecosystems that have been locked under the ice for thousands of years. The characterisation of these soils is important for our understanding of the cycling of organic matter under extreme environmental and nutrient limiting conditions, and their potential contribution to global biogeochemical cycles. This is particularly important as these new areas will become more geographically expansive with continued ice retreat.

Soil development following fresh exposure has typically been characterised through a chronosequence approach (space for time substitution) in which soils are analysed along a transect for changes in microbial community dynamics and biogeochemical element cycling. Here, for the first time, we present a new model that can simulate the initial stages of soil development following ice retreat. The model (SHIMMER: Soil biogeopHysIcal Model of Microbial Ecosystem Response) captures, explores and predicts the growth of various types of microbial biomass and transformations in carbon, nitrogen and phosphorus.

The model was tested and validated using published data from the Damma Glacier forefield, Switzerland (Bernasconi et al. 2011). We could easily predict the rapid accumulation of microbial biomass that was observed during the initial stages of microbial succession in the forefield . Furthermore, we show that primary production was responsible for the initial build-up of substrate that subsequently supported heterotrophic growth. Microbial production in young soils is supported by labile substrate, whereas carbon stocks in older soils are more refractory. Nitrogen fixing organisms are responsible for the initial accumulation of available nitrates in the soil. However, microbial processes alone do not explain the build up of organic matter observed in the data record. Consequently, the model infers that allochthonous deposition of organic material may play a significant contributory role that could accelerate or facilitate further microbial growth.

SHIMMER complements existing studies that use field data to describe the changing microbial communities and nutrient dynamics along the length of a chronosequence, providing a more quantitative evaluation on the dynamics of the system with a process-focussed approach, that has previously only been explored through qualitative interpretation of datasets.

The model framework is intended to be transferable to the initial stages of microbial commuity development in a range of environments from glacier forefields to ice sheet surfaces and desert soils and is currently being tested with data from a complex Arctic chronosequence from Svalbard.

KEYWORDS: DEGLACIATED FOREFIELD SOILS, MODELLING, CHRONOSEQUENCE

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Poster D-02

CHARACTERIZATION OF THE DAMMA GLACIAL MICROBIOME

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Glaciers are retreating worldwide due to global climate change. In Europe, alpine glaciers lost about half of their total surface area and their total volume within the last 150 years. Consequently, fresh rock sediments are exposed at the surface and subjected to weathering processes. Rock surfaces that are freshly exposed to the atmosphere become rapidly colonized by microbial communities as the first settlers. Despite the importance of microbial pioneers, their sources and identity are yet to be addressed. During soil development, microbial biomass and activity increase and microbial communities shift along soil surface. However, microbial succession is not just skin-deep.

A 454-pyrosequencing approach was used to assess whether bacterial and fungal community structures differed between stages of soil development (10, 60, 80, 110 years) and depths (surface, 5 cm, 20 cm depth) along a soil chronosequence (Damma glacier forefield, Switzerland). In parallel to plant establishment, carbon and nitrogen contents increased with soil age, particularly in the surface, leading to higher bacterial activity and fungal biomass in vegetated soils. Soil age significantly affected the bacterial and fungal community structures. Based on indicator species analyses, metabolically versatile bacteria (e.g. *Geobacter*) and psychrophilic yeasts (e.g. *Mrakia*) characterized the barren soils. Vegetated soils with higher C, N and root biomass consisted of bacteria able to degrade complex organic compounds (e.g. *Candidatus Solibacter*), lignocellulolytic *Ascomycota* (e.g. *Geoglossum*) and ectomycorrhizal *Basidiomycota* (e.g. *Laccaria*).

Soil depth only influenced bacterial and fungal communities in barren and sparsely vegetated soils. These changes were partly due to more silt and higher soil moisture in the surface. In both soil ages, the surface was characterized by OTUs affiliated to *Phormidium* and *Sphingobacteriales*. In lower depths, however, bacterial and fungal communities differed between stages of soil development. Lower depths of sparsely vegetated soils consisted of OTUs affiliated to *Acidobacteria* and *Geoglossum,* whereas depths of barren soils were characterized by OTUs related to *Gemmatimonadetes*. Overall, plant establishment drives the soil microbiota along the successional gradient but does not influence the vertical distribution of microbiota in recently deglaciated soils.

KEYWORDS: MICROBIAL DIVERSITY, GLACIER FOREFIELDS, NEXT-GENERATION SEQUENCING, PROKARYOTES AND EUKARYOTES

Poster D-03

ARCTIC AND ANTARCTIC SUPRAGLACIAL BACTERIAL DIVERSITY REVEALED BY NEXT GENERATION METAGENOMICS

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Scientific interest in the biology of glaciers was limited up to the 1980s because they were considered to be 'ice deserts' with little biological activity (Edwards et al. 2014a). Epifluorescent microscopy, cultivation and community fingerprinting techniques have been employed since then to analyze community dynamics in supraglacial environmental samples, exposing variability of bacterial taxonomic composition within those glacier habitats and between glaciers of different geographical locations (Cameron et al. 2012, Stibal et al. 2006, Christner et al. 2003). However, those approaches left the researchers unsatisfied. Metagenomics, in recent years, has provided more insight into microbial communities by sequencing microbial DNA extracted directly from environmental samples. Next generation sequencing has been used to assess the microbial diversity of Alpine and Arctic Glaciers (Edwards et al. 2014b). However, analogical data for Antarctic glaciers is still scarce. The analysis of available information allowed us to hypothesize that the diversity of supraglacial bacteriocenosis is dependent on geographical location as well as local conditions around the glacier and on its surface. To test this hypothesis a transect was established across the ablation zone of one Antarctic (Ecology Glacier, King George Is.) and two Arctic glaciers (Hans and Werenskiold Glaciers, Spitsbergen Is.), from the glacier terminus, up to the snow line. A representative quantity of surface ice was samples from 3 points per glacier. DNA was extracted from filter-concentrated samples and a 900 bp long fragment of bacterial 16S rRNA gene (covering 16S variable regions V1-V4) was amplified and sequenced using Roche 454 GS FLX+ platform. Sequences were identified using RDP Classifier website. Phylum level identification of obtained sequences revealed presence of several taxa in all three examined glacial surfaces, including: *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Acidobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Chloroflexi*, *Deinococcus-Thermus*, *Planctomycetes* and *Armatimaonadetes*. Major differences were observed between Arctic and Antarctic samples, like the scarce amounts of *Cyanobacteria* sequences in Ecology Glacier samples. Genus-level characterized sequences were subjected to statistical analysis to investigate the variability within the ablation zone. Shannon Index and the Effective Number of Species factor showed that in the case of Ecology (Antarctica) and Werenskiold Glacier (Arctic) the bacterial diversity was highest near the snow line and gradually decreased towards the terminus, whereas on Hans Glacier surface, the opposite was true. Three phyla constituting substantial portions of the bacterial community in all three glaciers (*Proteobacteria*, *Bacteroidetes* and *Actinobacteria*) were revealed to hold the same genera as their major components (*Polaromonas*, *Ferruginibacter* and *Frigoribacterium* respectively). Our findings allow us to conclude, that the supraglacial bacteriocenosis differs from one glacier to another, not only between hemispheres but also between glaciers originating from the same ice cap. Furthermore, the variability of bacterial taxonomic diversity is visible within the ablation zone of a particular glacier. The melting snow as well as "dry" aeolian inputs (especially at the glacier terminus) are responsible for local diversity increase. However, some bacterial genera were found in large quantities in all analyzed samples. These conclusions are consistent with the dual mechanism of microbe delivery to the glacier surface, specifically precipitation-associated (snow), and aeolian-associated (dust) (Xiang et al. 2009). Such deposition mechanisms and post-deposition selection are possible drivers of microbial community composition in glacial systems.

KEY WORDS: METAGENOMICS, SUPRAGLACIAL HABITATS, BACTERIAL DIVERSITY

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Poster D-04

THE BIOGEOGRAPHY OF CRYOCONITE BACTERIAL COMMUNITIES ON A HIGH ARCTIC ICE CAP

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Microbes of the supraglacial layer of glaciers have become a primary interest as they are now known to have a great effect on glacial ecosystems. Cryoconite microbial biomass is typically an autotrophic ecosystem. Uncovering the biodiversity and elucidating the biotic and abiotic factors that influence this habitat therefore becomes imperative to understanding the environmental impact of this unique habitat. This investigation describes the cryoconite bacterial community composition of Foxfonna (78˚ 08'N, 16˚ 07' E), an ice cap located in the archipelago of Svalbard. Ice caps are terrestrial ice masses which override the underlying topography, therefore the environmental gradients formed are principally the result of locally differential mass balance. The total community DNA extracted from 39 cryoconite holes distributed across the ice surface was used in V1-V3 16S rRNA gene amplicon semiconductor sequencing (Ion-Torrent Life Technologies USA). This was then analysed by QIIME for *de novo* OTU clustering and taxonomy classification before performing statistical analyses. The dominant phylum in Foxfonna Dome cryoconite appears to be *Proteobacteria* which contributes 28.3% towards the total bacterial community; within this, the class *Betaproteobacteria* is the most abundant contributor at 15.9% (Figure 1). This is closely followed by *Actinobacteria* with a community abundance of 21.84% and Cyanobacteria at 18.4%, with *Leptolyngbya* dominating. Distance based redundancy analysis (Figure 2) of species abundance displays that the role of slope and location (UTM N/E based on geographic location) are significant in Foxfonna cryoconite hole diversity. ANOVA on total OTU output supports this with highly significant effects for location, and chlorophyll on overall cryoconite hole diversity. All key phyla observed in Foxfonna Dome cryoconite holes are known to be dominant in the cryosphere. *Betaproteobacteria* are well known r-stategists that are capable of responding to environmental changes, which may explain their abundance. The level of significance observed in sample location and chlorophyll content, implies that the Foxfonna ice cap is dependent upon its location at elevated geographic levels as well as each holes intrinsic photosynthetic potential in order to maintain the dynamic communities in each cryoconite hole. Estimations of net ecosystem productivity from laboratory microcosm experiments have indicated that cryoconite bacterial communities are typically net heterotrophic under irradiances representative of in situ conditions; however the extent of net heterotrophy decreases with time. These phylogenetic and respiration results observed within individual cryoconite holes allow the inference that there exists a biological hierarchy within cryoconite holes, where cold adaptive and cold tolerant heterotrophs are the dominant taxa that are reliant upon photoautotrophs for nutrient control.

KEYWORDS: CRYOCONITE, ION TORRENT, 16S RRNA, MICROBIAL BIODIVERSITY

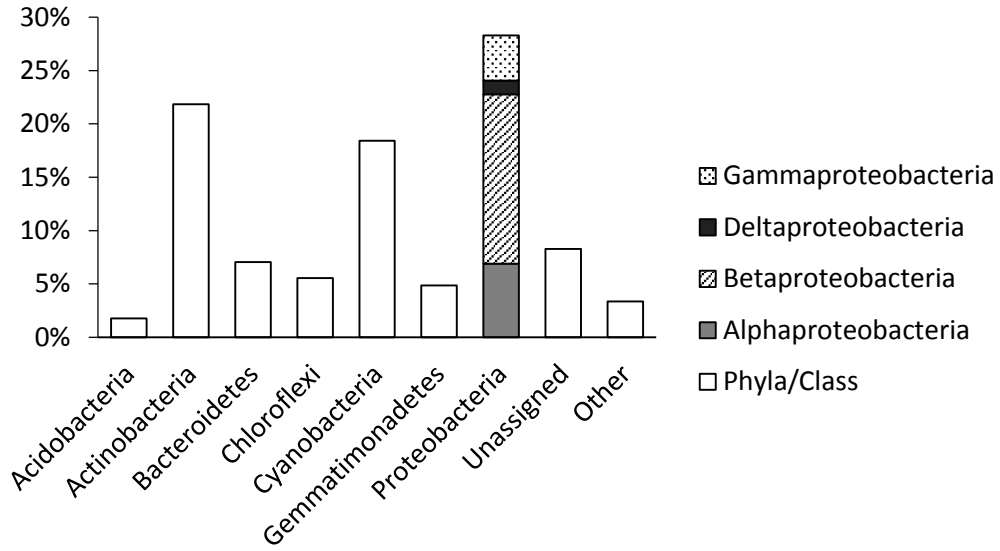

Fig. 1. Phylogenetic diversity based on OTU classification using QIIME with GreenGenes taxonomy.

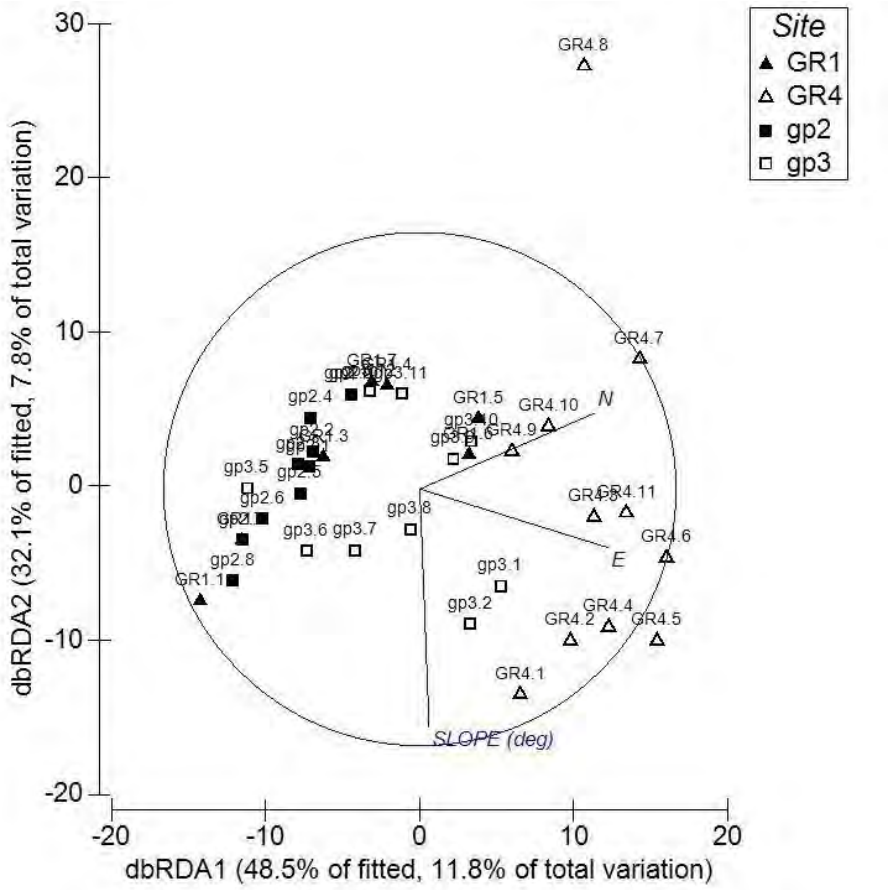

Fig. 2. Distance based redundancy analysis of Foxfonna Dome displaying geographic location (in UTM) and slope as important factors in community development.

Poster D-05

BACTERIAL COMMUNITY COMPOSITION IN VARIOUS SUPRAGLACIAL HABITATS OF ECOLOGY GLACIER (KING GEORGE ISLAND, ANTARCTICA)

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Glacier surfaces have been known to harbour diverse microbial live (Hodson et al. 2008). During the summer season those surfaces experience ablation phenomena, as the winter snow cover gradually recedes upwards. On those exposed ice zones, subjected to allochtonic inputs and heavy melting, crevasses, supraglacial streams and water reservoirs are created, being potential habitats of microbial life. Therefore, we hypothesize, that those different forms found on a glacier surface are inhabited by a unique set of microorganisms, which numbers and diversity depend on the prevailing conditions. The subject of this study were supraglacial habitats of Ecology Glacier (surface ice, snow, cryoconie holes and watercourses), located at King George Island, Antarctica.

Prokaryotic cell abundance and diversity were assessed by means of epifluorescent microscopy (DAPI staining and fluorescent in situ hybridization techniques). FISH probes were targeted towards α-, β-, γ-Proteobacteria, most gram positive bacteria, Actinobacteria, Cytophaga-Flavobacterium and Cytophaga-Flexibacter-Bacteriodes. Total number, biomass, biovolume and Shannon's index of prokaryotic cells were identified. Chemical parameters such as amounts of suspensed solids (SS), chlorophyll *a*, total and dissolved organic carbon (TOC, DOC) and basic nutrients (P and N) were used as the background to the study. All data were statistically processed.

Samples from Ecology Glacier differed in organic matter content and basic nutrient forms. Higher mean values of most chemical parameters were recorded in cryoconite holes. Significantly higher values of total nitrogen, phosphorus, chlorophyll a, iron and seston amounts were observed in samples at the top of the ablation zone, where the snow cover prevailed.

The total number of prokaryotic cells (TCN) in the analyzed samples ranged from 8.1 $x10^3$ to 1.95 x 10^6 ml^{-1} and varied depending on the microhabitat. The highest cell numbers occurred in cryoconite holes with an average of 7.48 $x10^5$ ml^{-1}. The surface ice cell numbers increased with the distance from glacier terminus and ranged from 8.1 x 10^3 (near terminus) to 1.0 x 10^5 (over 1km from terminus), with the average of 3.28 x 10^4 mL^{-1}. In snow samples TCN in 1 ml ranged from 1.5 x 10^4 to 5.2 x 10^4 ml^{-1} and a mean of 3.56 x 10^4. In the surface watercourses TCN was lower - an average of 2.0 x 10^4 ml^{-1} than in subglacial streams where the average cell count was 2.47 x 10^5.

In surface ice samples, the G+ bacteria group constituted 10.3% (including Actinobacteria - 4.1 %) of all prokaryotic cells, followed by α- and β-Proteobacteria (5.7%) and γ-Proteobacteria (3.6%). In snow samples G+ bacteria were most numerous (21% of all cells), Cytophaga-Flexibacter-Bacteriodes constituted 7.5% and β- Proteobacteria - 11%. In cryoconite holes β- Proteobacteria were less well represented (8.6%) and Cytophaga-Flexibacter-Bacteriodes constituted 7.6%. In the streams Cytophaga-Flavobacterium accounted for 22% of bacteria, and β-Proteobacteria 4.7%. In the water courses the least numerous were γ-Proteobacteria 0.6%.

Our studies show that the habitat conditions determine the phylogenetic composition of supraglacial bacteriocenoses. Snow and cryoconite holes determine the presence of Gram + bacteria, Proteobacteria and Cytophaga -Flexibacter-Bacteroides. Ice determines the occurrence of Actinobacteria and γ-Proteobacteria. Sub-glacial waterways determine the presence of Cytophaga-Flavobacterium.

Canonical analysis indicated a relationship between sampling point positions (distance from glacier terminus) and microorganisms occurrence. Microbiological parameter-based grouping of Ecology Glaciers supraglacial habitats showed that the cryoconite meltholes formed a single clade, the surface watercourses and sub-glacial outflows were separated and snow and ice clustered together to form smaller groups.

We conclude that each of the different glacial micro-habitats harbors a bacteriocenosis of unique taxonomic composition. Selection processes being responsible for the formation of this composition are the result of specific physico-chemical conditions occurring in these habitats.

KEYWORDS: BACTERIAL COMMUNITY COMPOSITION, FISH, ECOLOGY GLACIER, SUPRAGLACIAL HABITATS

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Poster D-06

MICROBIAL COMMUNITY CHANGES ALONG THE ECOLOGY GLACIER ABLATION ZONE (KING GEORGE ISLAND, ANTARCTICA)

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Microbiological investigations of glacial habitats have revealed that ablating glacier ice areas worldwide may harbor 1 x 10^{26} bacterial cells in the uppermost 1-2 m (Irvine-Fynn et al. 2012) and their microbial communities are active in biogeochemical transformations because they can perform metabolic functions involved in the C, N and P cycles (Hodson et al. 2007, Telling et al. 2011, Stibal et al. 2008). The glacial surface is annually covered by fresh snow whose melting during summer months exposes the ice below. As summer progresses, the ablation zone expands as the 'snow line' advances up the glacier (Hooke 2005). We hypothesize that gradual exposure of ice under melting snow is one of the major factors causing spatial variations of chemical composition, bacterial taxonomic and microbial functional diversity, and abundance in surface ice and cryoconite holes. We report here on microbial communities on the surface of Ecology Glacier, a rapidly receding glacier on King George Island, South Shetland Islands, Antarctica. Parameters of surface ice and cryoconite holes measured along a transect across the ablation zone included chemical composition of ice and sediment, *Bacteria* diversity by denaturating gradient gel electrophoresis (DGGE), microbial functional diversity (Biolog Ecoplates), and microbial counts according to colony forming units (CFU) and epifluorescence microscopy. We analyzed the data using Simple Regression and Principal Component Analysis (PCA) in order to identify environmental controls of microbial processes.

Nutrients (C, N, P), particulate matter (seston) and chlorophyll concentrations in surface ice and cryoconites were elevated near the snow line. Microbiological parameters of heterotroph and photoautotroph abundance and bacterial taxonomical diversity also displayed highest values in this area. These values declined gradually towards the glacier terminus, where the colony forming unit to total count ratio was the highest. As this ratio is considered a opportunism indicator in microbial communities (Siegler et Zeyer, 2004), it suggest more unstable conditions in this part of the glacier. Simple regression analysis pointed out several relations between chemical and microbiological parameters. The most notable were positive correlations between microbial abundance and diversity values and particulate matter content. The PCA indicated large differences between the surface ice and cryoconite habitats, especially in nutrient concentration and microbial numbers.

The melting snow proved to be a major source of nutrients and microbiota for the investigated supraglacial habitats, although the enriching effect was temporary. Cryoconite hole and surface ice communities develop and respond similarly to snow melt and exposure to allochtonic inputs, despite their differences in microbial numbers and diversity.

KEYWORDS: SUPRAGLACIAL HABITATS, MICROBIOCENOSIS, CRYOCONITE HOLES

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Poster D-07

SURVEY OF THE GLAICER INVERTEBRATES AND THEIR GUT MICROBIOTA

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In recently decades, biological investigations on glaciers have extensively been conducted; nevertheless, the knowledge on the invertebrates adapted to the glacier environment is still limited. Since glacier invertebrates are the predominant consumers on glaciers, it would be important to take their activity into account for the comprehensive understanding of the glacier ecosystem. In addition, it is conceivable that these invertebrates have a unique evolutionary history to adapt to glaciers, an extreme environment, therefore they would provide us insights into the mechanism and process of adaptive evolution. To understand the ecology and evolution of these glacier invertebrates, we have been focusing on their gut microbial symbionts. Recent studies have suggested that gut microbiota have great influence on the host activity and ecosystems through nutrient supply, immunity and material cycles. Given that the glacier is an extreme environment (i.e. cold, low nutrient and low biodiversity), the symbiosis between glacier invertebrates and gut microbiota would largely contribute to the adaptation of invertebrates to the glacier environment and the material cycle in the glacier ecosystem.

In the present study, in order to obtain basic information on the symbiosis between glacier invertebrates and microbiota, we analyzed the taxonomic compositions of bacteria physically associated with the glacier ice worm *Mesenchytraeus solifugus* and glacier stonefly *Andiperla willinki* based on the 16S rRNA gene sequences. DNA was extracted from the entire body or gut of each specimen, and 16S rRNA genes were amplified by PCR, cloned, and sequenced. We compared the similarities of bacterial community structure among the samples including their habitat glacier surface by UniFrac analysis. Additionally, we identified the localization of several dominant phylotypes in the host's gut by using fluorescence in situ hybridization (FISH).

We found that the majority of the obtained sequences were affiliated with known psychrophilic bacterial genera. This implies that the glacier invertebrates had built symbiotic relationships with bacteria indigenous in the glacier during the course of evolution. On the other hand, several 16S phylotypes were related to known symbiotic (i.e. gut-wall-associated or endosymbiotic) bacteria, which have never been detected from glacier environments. We found that some of these phylotypes were located on the gut wall of the glacier invertebrates by FISH. These results suggest that several symbiotic bacterial lineages have kept associations with the host invertebrates and co-adapted to the glacier environment. The UniFrac analysis showed that the bacterial community structures considerably differed between the gut microbiota and the surface of the habitat glaciers. This implies that there are unique niches inside the gut of glacier invertebrates distinct from the glacier surface (e.g. nutrient concentration and oxygen availability).

Our results provided the important suggestions for the adaptation and ecological niche of glacier invertebrates. We are currently performing the metatranscriptomic analyses of both glacier invertebrates and their associated microbiota to investigate the detailed relationship between them. I will include preliminary results of these analyses.

KEYWORDS: INVERTEBRATE, GUT SYMBIOSIS, 16S rRNA, TRANSCRIPT

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Poster D-08

COVER UP – COVERAGE OF GLACIAL SURFACES WITH INDUSTRIAL FLEECE TO REDUCE ABLATION: ECONOMIC BLESSING OR ECOLOGICAL SPELL? A SYMBIOSIS OF SOCIETY AND SCIENCE

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Climate change is affecting the alpine space which results in endangering the year round usage of ski slopes of glacial areas. So far, all glacial skiing areas in Tirol are threatened by large losses of ice masses if they would not take action to reduce ablation which can be achieved by coverage of large areas with industrial fleece. The fleece is made of polypropylene and can help to preserve ca. 1,5m of snow during the coverage period from May to September. The economic gain is evident, however, there are serious ecological concerns regarding the usage of this fleece on snow. Snow and ice are inhabited by living communities consisting of microbes and metazoa which show an active metabolism depending on the availability of liquid water. Consequently, with coverage during the main vegetation period a change in numerous living conditions is the case, i.e. the incoming radiation which is crucial for autotrophic organisms. The most critical issue is the addition of a lubricant which is water soluble. During melting phases and precipitation events this lubricant will be dissolved from the fleece and potentially will not only affect the living communities concerning biodiversity and activity but also the water circuit. It is the scope of this project to define and quantify the impact and implications of the fleece on the organisms in ice and snow. Moreover, we want to initiate a constructive discussion with stakeholders and producers of the fleece to possibly find alternatives to avoid the usage of lubricants. Due to a close collaboration with young students during field studies as well as laboratory work, this interdisciplinary approach should enable the next generation to achieve a sensitive perspective on our living space in the Alps and the touristic implications and effects on our environment. Our investigation area is located in the Stubai Alps, Austria (ca. 2400 m a.s.l). The industrial fleece covers test fields with various treatments which are laid out during the snow coverage period. Snow, ice and melting water samples of uncovered and covered areas are collected every other week to obtain an elaborate data set until final snow melt. To analyze abiotic and biotic parameters state-of-art methods will be used. In addition to an extensive biogeochemical investigation, we aim for a detailed biodiversity survey. Therefore we will have a look at bacterial and fungal abundance (DAPI staining, phase contrast), metabolic activity (Live-Dead stain), bacterial biomass (epifluorescence microscopy), autotrophic and heterotrophic productivity (Incorporation of $NaH^{14}CO_3$ and ^3H-leucine) as well as DNA extraction, PCR and subsequent sequencing for algae, fungi and bacteria to obtain phylogenetic analysis. Furthermore, we will investigate the effects of the water soluble lubricant on occurring microorganisms using cultivation experiments.
Relying on previous research, we are expecting significant differences between covered and uncovered glacier areas concerning their nutrient availability as well as microbial community composition. The lack of incoming radiation, nutrient deficiency and additional impurity with the potentially deleterious lubricant, may lead to a reduction of biodiversity as well as activity within such a sensitive ecosystem.

KEYWORDS: GLACIER COVERS, ABLATION CONTROL, CLIMATE CHANGE, LUBRICANT, INTERDISCIPLINARY APPROACH

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Poster D-09

SEASONAL CHANGES IN BIOLOGICAL ACTIVITY IN ICE-BOUND ECOSYSTEMS ON SIGNY ISLAND, MARITIME ANTARCTICA

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Continued warming of the maritime Antarctic is leading to expansive areas of snow and ice becoming increasingly biologically active, resulting in an increased flux of microorganisms and nutrients from snow and ice-bound habitats to the marine environment. Microbial abundance on the surface of glacier ice alone is estimated to be 10^{14}-10^{17} cells per km^{-3} making it the largest freshwater reservoir of microorganisms on Earth. Due to the recent rapid regional warming in West Antarctica and around the Antarctic Peninsula in particular, large masses of ice and snow have been lost into the surrounding ocean (~180 Gt of ice $year^{-1}$). With this ice, around 16 Gg of organic carbon are also released every year and transferred into the ocean, but the ecological implications of such loading for the marine ecosystem remain unclear.

We aim to assess climate forcing on biogeochemical activity in snow and ice-bound ecosystems in the maritime Antarctic and to estimate nutrient and biomass export by glacier meltwater into terrestrial habitats and coastal waters. Microbiology, nutrient economy and productivity of snow and ice surface habitats were assessed at two major glaciers on Signy Island (South Orkney Islands) in maritime Antarctica. These sites represent the broad range of melting and nutrient gradients found along much of the Antarctic Peninsula's west coast and associated archipelagos. Microbial community structure and biomass changes were studied in snowpack, slush and superimposed ice during the austral summer 2012-13 using molecular techniques (16S and 18S illumina MiSeq), pigment analysis and flow cytometry. Fluxes of carbon, nitrogen, phosphorus were also monitored throughout the season within the glacier ice and snowpack. Changes in net ecosystem production, respiration and photosynthesis of the snow and ice-bound habitats were evaluated using radioisotope labelling (^{14}C and ^{3}H) and in situ CO_2 flux measurements. This multidisciplinary approach enabled us to calculate the internal biological production and biogeochemistry of snow and ice-bound ecosystems on Signy Island and to estimate the significance of the nutrient and microbial loading from these melting icy habitats into the surrounding coastal ecosystems.

KEYWORDS: GLACIER, SNOW, MARITIME ANTARCTICA, MICROBIAL PRODUCTION, NUTRIENT CYCLING

Poster D-10

BACTERIAL DIVERSITY AND BIO-POTENTIALS OF HIMALAYAN CRYOCONITES, AND ITS COMPARISON WITH ARCTIC

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Owing to the fact that the Himalayan region comprises of the biggest mountain range on Earth and the largest ice mass outside the polar region, a need to access and compare its glacial cryoconites with the polar glacial cryoconites was realized. There is no comparative report on bacterial diversity of Cryoconites of Arctic and Himalaya is available for these geographically apart yet climatically similar regions of the world. The present study was therefore undertaken to get a comparative account of the bacterial diversity in the glacial cryoconites of the Himalayas and the Arctic.

Cryoconites are unique habitats in glacier environments and of ecological and biotechnological importance. The collected cryoconites were inoculated onto bacteriological media. Representative bacterial isolates were identified through 16S rRNA gene sequencing. *Bacillus*, *Microbacterium* and *Pseudomonas* were the most dominant genera, and the common species were *Acinetobacter lwoffii, Bacillus aryabhattai, Bacillus marisflavi, Bacillus foraminis, Bacillus humi, Cryobacterium arcticum, Chryseobacterium hispalense, Glacimonas singularis, Janthinobacterium lividum, Kocuria palustris, Micrococcus aloeverae, Microbacterium hominis, Micrococcus luteolum, Microbacterium paraoxydans, Pseudomonas psychrophila, Pseudomonas meridiana, Pseudomonas mandelii, Rhodococcus fasians, Rugamonas rubra,* and *Sphingobacterium multivorum.* Physiological, biochemical, antibiotic susceptibility and enzyme screening tests were carried out. Cold-adapted enzyme production ability of the bacterial isolates provides a possible prospect in biotechnological research. These data present the first record of culturable bacterial communities and their characterization from cryoconites of Himalayas.

Twenty five elements: Arsenic (As), Barium (Ba), Bismuth (Bi), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Cesium (Cs), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb), Lithium (Li), Rubidium (Rb), Strontium (Sr), Uranium (U), Vanadium (V), Zinc (Zn), Calcium (Ca) and Magnesium (Mg) were analyzed using Inductively Coupled Plasma Mass Spectroscopy (ICPMS). The data reveals that, the Himalayan glaciers accumulate many of the elements in concentrations higher than the Arctic.

The data reveals that, the Himalayan glaciers have high concentration of elements and bacterial diversity than the Arctic cryoconites. Rapid industrialization going on in the Asian countries could be a likely cause.

KEYWORDS: BACTERIA, HIMALAYA, ARCTIC, CULTURABLE DIVERSITY

Poster D-11

BACTERIAL DIVERSITY IN TROPICAL GLACIER AND GLACIER FORELAND IN UGANDA

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Tropical region is still missing area of glacier biology, though most studies on glacier ever had been reported frequently from mid latitude to bipolar region. In tropical regions glacier retreat is more significant, especially glaciers in Rwenzori Mts. where located between Uganda and Congo was expected to disappear within a decade. Otherwise, recently novel biological aggregation, which is mainly formed by moss gemmae, was found from glacier in Rwenzori Mts. (Uetake et al. 2014). This aggregation is not simple structure only by moss gemmae, but supply habitat for many glacier microorganisms. And after retreat of glacier terminus, abundant of GMGA are left on the fresh bared rocks and became soil like structure within few years.
In order to know the bacterial community structure changes after/during formation of GMGA structure and after release to glacier foreland, 16S rRNA amplicon from no GMGA covered ice surface (3 sites), GMGA (1 sites) and glacier foreland soil (6 sites) were sequenced by Miseq and sequence data set were analyzed by Qiime.
Principal coordinate plot of bacterial communities of GMGA is closer to glacier foreland samples, rather than no GMGA covered glacier ice. Genus Chloroflexi are most abundant in glacier foreland soil (15- 56% relative abundance: mean 34±15%) and GMGA (30% relative abundance), otherwise less in no GMGA covered ice surface (3- 6% relative abundance: mean 4±2%). Most of Chloroflexi are belong to family: Thermosporotrichaceae and Ktedonobacteraceae. Difference of bacterial communities may be caused by at least thickness of structure and relatively higher temperature than glacier surface.

KEYWORDS: GLACIER MICROBIOLOGY, TROPICAL GLACIER

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Poster D-12

INVESTIGATION OF THE PROGLACIAL ZONE AS A MODULATOR FOR NUTRIENT FLUXES IN ICE SHEET RUNOFF

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Microbial communities in supraglacial and subglacial environments play an important role in nutrient cycling and chemical weathering in the Polar Regions. While a growing body of data exists from small valley glaciers, there is very limited data on nutrient cycling in ice sheets systems. Recent research indicates that ice sheets may play an important role in global elemental cycles. A particular area of interest concerns their potential to export nutrients to downstream ecosystems. However, one of the major areas of uncertainty is the extent to which nutrients exported in ice sheet runoff may be modulated en route to the ocean via biogeochemical processes within the proglacial zone. This is an important consideration in an era of ice sheet retreat and expanding proglacial land surface areas. This poster presents data on the speciation and concentration of dissolved inorganic/organic macronutrients (N, P and Si) and their absorption onto particulate material during transit through the proglacial zone bordering the Greenland Ice Sheet. We focus on these macronutrients because of the tendency for one or more of them to limit phytoplankton growth in the Polar oceans. We measured changes in dissolved macronutrient concentrations along a proglacial reach at two field sites in South West of Greenland (Watson river, Kangerlussuaq) and South East of Greenland (Mittivakkat River, Sermilik), also monitoring temporal variations in the same species at the end of the reach. Results from these two proglacial surveys revealed no significant change in the macronutrient composition of the meltwater as it travels from the glacier mouth to the fjord, even over a distance of 25km. We infer that this reflects the relatively fast transit of meltwaters (estimated at 1 – 1:30 hr at each site) via major river systems in the proglacial zone. We, therefore, postulate that the glacier represents the most important source of nutrients into the near-coastal regions. We also conjecture that, provided flow paths remain channelized, an expanding proglacial zone in a warming climate may not significantly influence macronutrient concentrations and speciation exported in glacial runoff to the coastal ocean.

Poster D-12

GREENLAND ICE SHEET AS A MODEL FOR MICROBIAL MACROECOLOGY AND EVOLUTION

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INTRODUCTION

The ancient ecological question - Why are some organisms found here and not somewhere else? – is not so easy to answer when it comes to microorganisms due to the striking complexity of their communities. Understanding the spatial patterns in the distribution of microbial biodiversity on Earth is an effort requiring a combination of the fast developing methods of molecular microbial ecology and a theoretical framework into which the vast amount of data generated by these methods can be integrated. We present a progressive investigation of the microbial diversity patterns over multiple spatial scales in an environment dominated by microbial life and characterised by low environmental variability in space – the ablation zone of the Greenland Ice Sheet (GrIS).

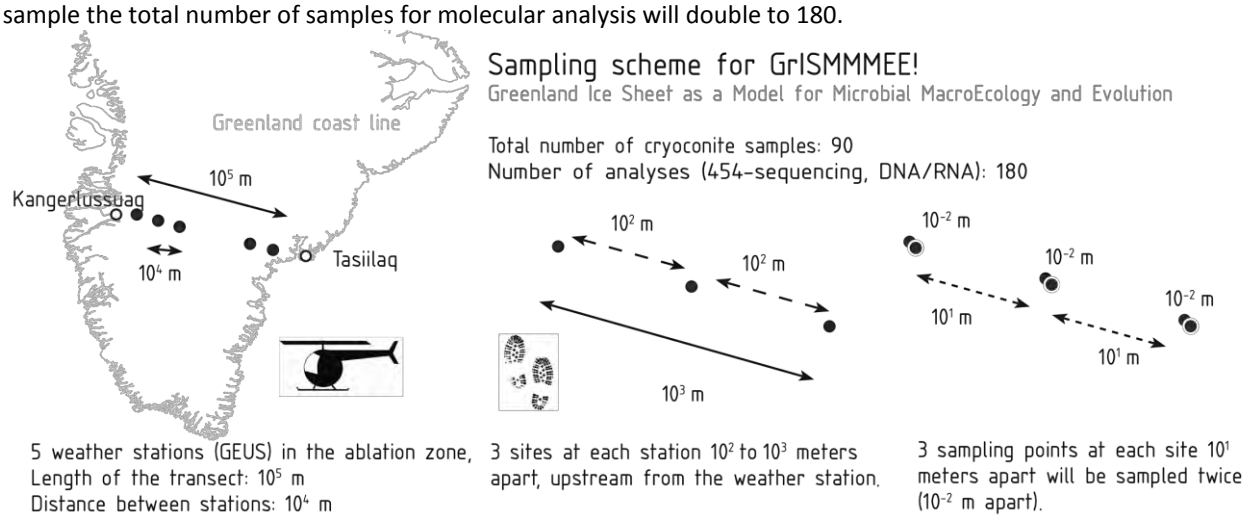
At the beginning of 21st century Finlay and Fenchel (Fenchel and Finlay 2004) brought a new impulse into the discussion about microbial biogeography, which has its roots in the Baas-Becking hypothesis "everything is everywhere" and "environment selects". Their conclusions have been discussed from different perspectives of protistology and macroecology (Green and Bohannan 2006), and they still play an important role of a 'nucleation agent' for conceptual interactions among specialists from different subject fields.

There is an on-going discussion on the spatial patterns of microbial diversity, fuelled by findings suggesting different biodiversity patterns for plants and microbes and the importance of a phylogeographic approach for elucidating the processes shaping them (Bryant et al. 2008). These efforts have brought forward the role of spatial patterns of diversity in the microbial realm, and identified the need to use different spatial scales for microbial ecology research (Green and Bohannan 2006). Therefore, we place a strong emphasis on covering a broad range of scales (see Sampling scheme, Fig. 1) at which we will investigate spatial patterns of microbial diversity.

The Greenland Ice Sheet (GrIS) is the second largest ice sheet in the world (1.7 million km^2). Its surface is relatively spatially homogeneous (as opposed to soils), it is not subject to large-scale mixing (as opposed to seawater), and most environmental variables affecting the microbial community on the glacier surface (such as nutrient concentrations) can be assumed to be co-variables of altitude or distance from the glacial margin, respectively (Stibal et al. 2012, Anesio and Laybourn-Parry 2012). Therefore, the surface of the GrIS represents an excellent model for testing macroecological hypotheses in a microbially-dominated ecosystem.

MATERIAL AND METHODS

The suggested sampling setup will contain nested spatial scales from 10^5 to 10^{-2} m. The total distance across the ice sheet is in the range of 10^5 m, with distances between stations on the transect being in the range of 10^4 m. At each station three sampling sites with 10^2 m spacing (10^3 m between 1st and 3rd site) will be selected, and at each site three points (10^1 m apart) will be sampled in parallel (10^{-2} m apart, Fig. 1). This will result in the collection of 90 samples covering the scale from 10^5 down to 10^{-2} m. Due to the co-extraction of RNA and DNA from each sample the total number of samples for molecular analysis will double to 180.



Compositional and phylogenetic similarity between all pairwise comparisons of communities will be quantified with the Sørensen Index and PhyloSor (Bryant et al. 2008), respectively. By analogy with the well established distance-decay relationship which describes the decrease in compositional similarity between two communities with increasing geographic distance, the PhyloSor will be used to quantify the decrease in phylogenetic similarity with distance (phylogenetic distance-decay). Using PhyloSor we will test whether two communities are more or less similar than expected given their taxa similarity. This will be done by comparing the phylogenetic similarity of the observed communities to a null expectation obtained by randomly sampling the pool of all of the species identified in the study while constraining the number of taxa in each community and the number of taxa shared in each community. We will test whether the slope of the decay of phylogenetic similarity is greater or lower than what was expected given the taxonomic decay in similarity by comparing the observed slope with a distribution of distance-decay slopes obtained by randomizing the location of taxa. Our preliminary data from 2013 will be used as a pilot study for further investigations.

KEYWORDS: GREENLAND ICE SHEET, BETA DIVERSITY, DIVERSITY PATTERNS

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Session E
Polar/alpine cyanobacteria

Keynote lecture KN-E

POLAR/ALPINE CYANOBACTERIA

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Cyanobacteria represent one from the most common and most important components of phototrophic microorganisms in both polar regions. They occur and form cyanobacterial populations and characteristic and dominant communities, particularly in various habitats in deglaciated areas. The cyanobacterial populations are dominant in microbial communities of seepages, streams, in littoral and benthos of lakes, and on the wetted soil surface in the form of crusts. Structure and species composition of these assemblages in main habitats is more or less characteristic for different ecosystems and stable in time, during the following years. Several special cyanobacterial types occur as dominant in such ecosystems, but they were not registered outside polar regions in their typical form. Their phytogeographic specificity is combined very closely with ecological demands of various genotypes. Up to now, they were not found any ubiquitous taxa. On the other hand, cyanobacterial taxa adapt strictly to certain changed conditions and the occurrence of limited ecotypes (often combined with morphological adaptations) is commonly known. From this situation follow the main tasks of the investigation of cyanobacteria in polar region:

1) Precise taxonomic characterization and identification of main cyanobacteria in polar habitats, especially of dominant types in polar communities; they must be determined according to polyphasic methods, and identification of their autecology and life cycles are necessary.
2) Identification of specific geno-, morpho- and ecotypes, characteristic exclusively for polar habitats (identification of respective endemic species). The study of their ecology and function.
3) Identification of respective similar communities in habitats outside Antarctica; their comparison, especially the species dominancy and composition.
4) Identification and characterization of possible genotypes typical for polar communities, and their comparison with the related populations outside polar regions, which are similar according to morphology or by genetic criteria (e.g., by molecular analyses or sequencing).
5) The study of the role of transported cyanobacterial genotypes in polar ecosystems, their long-term survival and their function.

KEYWORDS: CYANOBACTERIA, POLAR REGIONS, IDENTIFICATION, DISTRIBUTION, ECOLOGY

Lecture E-01

DIVERSITY OF HYPOLITHIC CYANOBACTERIA FROM THREE LOCATIONS IN WESTERN SPITSBERGEN

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Auto- and heterotrophic communities that colonize the soil-imbedded surface of translucent stones are named 'hypolithic' or 'hypoliths'. Especially in arid and semi-arid regions, where vascular plants are less abundant, hypolithic organisms are of importance for biomass and productivity. These drylands comprise more than 30% of Earth's land surface and hence hypoliths have been found almost everywhere in these regions. The dominance of phototropic cyanobacteria in the hypolithic community is a shared feature of all drylands. Earlier research mostly focused on non-polar desert communities in North and South America, Africa, Asia and Australia. However, hypoliths of polar deserts gained only recently attraction. Previous studies mostly concentrated on the Canadian Arctic, Tibetan tundra and Antarctic areas.

At the Arctic polar archipelago Svalbard, cryptogams (algae, lichens, fungi and mosses) and Cyanobacteria dominate the vegetation. A number of earlier biodiversity studies focused on terrestrial (tundra soil, moss tundra), freshwater (glacial and tundra streams, pools), and seawater habitats. However, no study focused on hypoliths in that region. The aim of this study is to gain preliminary data on hypolithic cyanobacterial communities from West-Spitsbergen, Svalbard. Samples were collected in August 2014 from three different localities characterized by frost polygons with medium-grained soils near Ny-Ålesund. At each side a number of translucent stones have been collected (in total 26 samples) and transported to the lab for further analysis. Attached biomass was transferred to solidified BG11 medium (1.5 %) and cultivated at 12-15°C and ~20 µmol photons $m^{-2}s^{-1}$ for about 2 months. Species were morphological identified according to taxonomic literature, photographed and measured using light microscopy. Potential new species for Svalbard have been further phylogenetically analyzed and compared with known species.

The results of this study revealed that coccoid and filamentous Cyanobacteria of the subclasses Synechococcophycidae, Oscillatoriophycidae and Nostocophycidae are present in theses terrestrial niches. Diazotrophic species were also found (e.g. *Gloeocapsa, Nostoc* and *Tolypothrix*). Some species developed thick sheaths able to glue soil particles together and thus stabilizing the soil. The composition of the hypolithic community at Spitsbergen indicate a higher biodiversity than communities described from other polar deserts.

KEYWORDS: CYANOBACTERIA, HYPOLITHS, POLAR DESERT, ARCTIC, BIODIVERSITY

Poster E-01

THE EVOLUTION AND DIVERSITY OF CYANOBACTERIA IN THE CRYOSPHERE

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Cyanobacteria are major primary producers in the polar and alpine regions contributing significantly to nitrogen and carbon cycles in the cryosphere. However, the processes by which cyanobacteria capable of survival in cold environments first evolved are not fully understood. Here we present an overview of cyanobacterial diversity in polar and alpine environments and use ancestral state reconstruction to show that many lineages of cyanobacteria shared cold tolerant ancestors. The occurrence of shared ancestors to geographically disparate lineages has considerable implications for the evolution and biogeography of cold tolerant cyanobacteria and may help us to understand methods underlying their radiation into cold environments.

Poster E-02

CHARACTERIZATION OF TEN STRAINS OF FILAMENTOUS CYANOBACTERIA FROM THE SOUTH SHETLAND ISLANDS, MARITIME ANTARCTICA

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The evolutionary relationships of ten Antarctic cyanobacterial strains of the order Oscillatoriales isolated from King George Island and Deception Island, South Shetland Islands were studied by a polyphasic approach. Phenotypic observations of the morphological features and genotypic analyses (16S rRNA and ITS sequences) were performed. Based on major phenotypic features, the strains were divided into four distinct morphotypes: *Leptolyngbya borchgrevinkii* (A)*, Leptolyngbya frigida* (B), *Phormidium autumnale* (C) and *Wilmottia murrayi* (D). This morphological identification was in global agreement with the evolutionary relationships. According to the phylogenetic analysis, the ten strains were divided into two major clades, containing related strain sequences with *Leptolyngbya* morphotypes in one clade and with morphotypes corresponding to *Phormidium, Wilmottia* and *Microcoleus* spp. in the other clade. Each major clade was divided into two sub-clades. For the first time, the 16S rRNA gene sequence of a strain corresponding to the *Leptolyngbya borchgrevinkii* morphotype (A) was determined, on the basis of strain KOVACIK-ANT 1990/4. The closest sequence to our morphotype A is the clone Fr252 isolated from microbial mat of Antarctic Lake Fryxell. Morphotype B is closest to sequences assigned to *Leptolyngbya frigida* isolated from microbial mats of lakes in continental East Antarctica. Morphotype C belongs to a cluster including strains with morphotypes corresponding to *Phormidium autumnale* from Antarctica, but also from Europe. Morphotype D is grouped with sequences of the morphotype assigned to *Wilmottia murrayi* isolated from Antarctica.

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Poster E-03

MORPHOLOGICAL AND MOLECULAR CHARACTERISTICS OF *Nostoc commune* VAUCH. EX BORN. & FLAH. POPULATIONS IN MOUNTAIN AND ARCTIC HABITATS

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The investigation of cosmopolitan cyanobacteria *Nostoc commune* is of great significance since the specie holds an important position in structural and functional organization of terrestrial ecosystems due to its ability to fix molecular nitrogen. In polar regions and extreme habitats, *N. commune* is a dominant specie among spore plants. Moreover, the species is distributed world-wide and lives under high variety of ecological conditions, *N.commune* is widely used as model specie by many scientists (Novis et Smissen 2006, Reháková et al. 2007, Arima et al. 2012). The morphology and ecology of the specie have been studied properly and authors point out its high polymorphism: the presence of several morphological forms is known. However, the reasons for such high polymorphisms fo rspecie are still unknown since data on genetic variability of *Nostoc* in different habitats and regions is rare, especially, for Russian North and Arctic.

The study was conducted to examine variation within cyanobacteria *N. commune* collected in arctic and mountain habitats. The aims of the project were to collect samples and isolate them into culture, describe morphological features of the colonies and cells, to study genetic variation using 16S rRNA sequencing and AFLP methods. Samples were collected during 2000-2012 on Svalbard, Polar and Subpolar Urals, Ridge Pai-Khoi, the North Caucasus and Baikal area. For the amplification of the 16S RNA fragments primers CYA781R and CYA106L were used. Sequence alignments and analysis was performed by program MEGA 5.0. Method of combining nearest neighbors (NJ) was used to construct phylogenetic trees. EcoRI-ACG/MseI-CTA, EcoRI-ACG/MseI-CTT, EcoRI-AGG/MseICTA, EcoRI-AGG/MseI- CTT, EcoRI-AGC/MseI- CTA, EcoRIAGC/ MseI-CTT и EcoRI-ACA/MseI-CAA primers were used for AFLP. Data analyzes was conducted using R and Structure v.2.3.4.

The colonies of *N. commune* varied in size, color, depending on availability of water. The smallest colonies up to 3 cm in diameter were observed on Svalbard, the largest colonies (20-30 cm) were registered for Polar Urals. The largest cells were from Polar and Subpolar Urals collections, and the smallest cells were found in North Caucasus and Svalbard. The largest heterocytes were registered in Polar Urals and Baikal, and the smallest were found on Svalbard and North Caucasus. Though the colonies have their own morphological characteristics, the cells and heterocytes size as well as mucliage or cell shape features did not correlate with latitudinal gradient. The small size of the colonies on Svalbard could be associated with extreme environmental conditions in the region. It is the northernmost location among the areas studied with the shortest growing season. Polar Urals colonies had maximum size, then probably, because they were collected on the outputs of carbonate rocks, the habitat is most favorable for growth of *Nostoc.* In general, for all regions the following pattern was observed: aquatic population excessed terrestrial, the size of the colonies differed markedly due to the development of huge polysaccharide mucous covers.

On phylogenetic tree, strains from different populations of European North (Polar, Subpolar Urals and Pai-Hoi) grouped in one cluster. The south populations of mountain and forest steppe (Caucasus, Baikal regions) formed the other. A strain from Svalbard was the most genetically distinct population which is probably connected to its island isolation. For detailed study of genetical variation within *N. commune* AFLP for 7 populations were conducted. The AFLP analysis did not reveal number of population which can be connected to high levels of within-species genetic similarity. For evaluation of genetic variation of *Nostoc* populations further investigations are needed. More ecological factors influencing *N. commune* should be taken into consideration of its genetic variability rather than latitude.

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KEYWORDS: *Nostoc commune* GENETIC DIVERSITY, CYANOBACTERIA MOUNTAIN AND ARCTIC REGIONS RUSSIA

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Poster E-04

DIVERSITY OF PHOTOTROPHIC BACTERIA INSCARISOARA ICE CAVE

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We here report on the structural diversity and metabolic activity of phototrophic bacteria from perennial ice sediments of Scarisoara Ice Cave, Romania, considering their remarkable ability to tolerate abiotic stress from cold environments. Ice samples were collected from direct- and indirect-sun exposed 1-year old ice, and light deprived 400 and 900 years old ice of the underground ice block. The presence of both cyanobacteria and algae was revealed by bright field and fluorescence microscopy, and cultivation on BG11 medium. Cell density was determined by measuring the chlorophyll absorbance and fluorescence. PCR-DGGE analysis and amplicon sequencing of the cyanobacterial-specific 16S rRNA genes were used to identify the diversity of cave ice-embedded phototrophic prokaryotes. 16S rRNA pyrosequencing of ice microbiota from 5 ice samples allowed the analysis of phototrophic taxa in recent and old cave ice. The occurrence of uncultured cyanobacteria throughout the ice block was revealed by 16S rRNA gene amplification, while only recent ice samples exposed to light contained cultivable phototrophs. Microscopy analysis indicated the presence of natural fluorescent, coccoidal and filamentous cells. SybrGreen (total cells) and ethidium homodimer (dead cells) labeling highlighted the presence of viable cells in the cave glacier. Transparent exopolysaccharides particles are present in all ice samples. The calculated growth parameters of recent ice microbiota cultivated in the presence of light strongly depend on the growth temperature. DGGE profile indicated a higher diversity of uncultured cyanobacteria relative to the cultured ones in all ice layers, and the presence of OTUs homologous to strains from cold and temperate environmental samples. Pyrosequencing data revealed high relative abundance of the anoxygenic Chloroflexi, Cholobi, and oxygenic Cyanobacteria phyla in sun- and light-exposed layers from the ice block surface, while present but more scarcely represented in 400 and 900 years old ice samples.

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KEYWORDS**:** ICE CAVE, PHOTOTROPHS, 16S rRNA GENE, DIVERSITY

Poster E-05

IMPACT OF ENVIRONMENTAL FACTORS ON SOIL CRUST COMMUNITY IN SVALBARD

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Biological soil crusts are important components of Arctic desert and semi-desert ecosystems and comprised of various combinations of biological communities including bacteria, cyanobacteria, microalgae, fungi, mosses, lichens and invertebrates. This study was aimed to compare abundance of soil crusts and their microbial community composition (primary producers PP -cyanobacteria, microalgae; consumers C - bacteria and fungi) and invertebrates together with their environmental controls (temperature, water content, pH, conductivity, total organic carbon, $N\text{-}NH_4$, $N\text{-}NO_3$, $P\text{-}PO_4$, Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) in three different habitat types. The sampling sites were located in the vicinity of the Petunia Bay, the north-western branch of Billefjorden, central part of Svalbard, in the following habitat types; a) Raised marine terrace (T) - area deglaciated at the beginning of the Holocene characterised by full vegetation cover with dominant plant community *Dryas octopetala* and *Carex rupestris* as co−dominant; b) Top of Mummien Peak (P) - deglaciation started from late of Pliocene, negligible plant cover is characterised by *Papaver dahlianum* community, and c) Foreland of Horbye Glacier (G) - recently deglaciated area - up to 10 years, without plant cover. We hypothesized that environmental parameters including time of deglaciation and microclimatic conditions greatly influence abundance and composition of soil crust community. Three years mean the most frequent ground temperature and the most frequent volumetric water content of upper soil layer differed remarkably (T site: 0°C = 35% and 0.1 $m^3\ m^{-3}$ = 59%, P site: -5°C = 36% and 0.1 $m^3\ m^{-3}$ = 69% and G site: -5°C = 34% and 0.05 $m^3\ m^{-3}$ = 58%), respectively. T site with plant cover was warm and wet, top of peak P with soil crust only was cold and wet, and glacier foreland G was cold and dry. Number of observed days with temperature above zero was 103 in site T, followed by 98 for site G and 87 days per year for site P. Total organic carbon and $P\text{-}PO_4$ contents were considerably higher in more developed soil in T site. The P site was richest in cyanobacteria-microalgae cell numbers followed by T site. The T site had the highest content of heterotrophic microbial biomass (based on PLFA pattern) as well as invertebrate abundance (based on microscopic counts). However, there was high site variability between PP and C ratio. PP showed significant positive canonical correlation with K, consumers - bacteria and fungi with pH, Ca, conductivity and invertebrates with $P\text{-}PO_4$, pH and Ca. Principal component analysis on composition of PP, C and invertabrate communities in studied habitat types showed differences in the soil crusts microbiota between and inside habitat types.

Poster E-06

THIN AND UBIQUITOUS: TAXONOMIC FEATURES OF GENUS *Leptolynbya* IN POLAR REGIONS

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Cyanobacteria belongs to major primary producers in the Polar Regions. Very thin non-motile filaments are found within every terrestrial and freshwater cyanobacterial assemblage. Such thin and non-motile cyanobacteria are commonly classified into genera Leptolyngbya (Anagnostidis et Komárek 1988). Cyanobacterial genus *Leptolyngbya* was created from thin species of old traditional genera *Lyngbya*, *Phormidium* and *Plectonema* (LPP). This genus is characterised by thin and non-motile filaments without heterocytes and parietal locations of thylakoids. Intergeneric classification of such specimens is extremely difficult for having a simple morphology. There were already described a relatively high number of endemic taxa (e. g. *Leptolyngbya nigrecsens, L. antarctica*). The accurate taxonomic designation and also ecological distribution of species within traditional genus *Leptolyngbya* would elucidate the role of cyanobacterial flora in polar aquatic and terrestrial biology.
In this study we analysed by polyphasic approach 38 *Leptolyngbya* strains (filaments with ± short cells, minimal width of cell below 3 µm and various properties of sheaths) originated from both Polar Regions. The approach includes morphological, ultrastructural and genetic (16S rRNA gene sequencing) analyses. The studied strains showed a remarkable morphological variability within their life cycle that is also induced by growth conditions (i.e. light intensity). Based on genetic properties the studied strains are split into 11 genera. However, the morphometric analysis revealed that studied strains should be split into 3 different phenotypes according the type of filaments: *Leptolyngbya*-like – created by quadratic or shorter then wide cells with one central granula; *Protolyngbya*-like – created by longer then wide cells with small granules on cross-walls and *Arthronema*-like – created with ± irregular cells without granules. The described combination of genetic and morphologic features of studied strains reveal complicated and taxonomically difficult complex of cryptic species.

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Poster E-07

A NEXT-GENERATION PROTOCOL FOR THE ASSESSMENT OF CYANOBACTERIAL DIVERSITY

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The field of microbial ecology has undergone a revolution with the advent of next-generation sequencing technologies, which revealed a higher microbial diversity than what was previously observed (Sogin et al. 2006). The possibility of analysing tens to hundreds of thousands sequences in a single sequencing run has provided information on rare taxa that could constitute an important fraction of microbial communities. However, this comes with the cost of relatively high error rates for individual reads, which can lead to overestimation of diversity due to the generation of spurious OTUs consisting of erroneous sequences (Kunin et al. 2010). Therefore, a correct assessment of microbial diversity using NGS relies on robust bioinformatic tools in order to correct for PCR and sequencing biases. This study was carried out to test different protocols for 454 pyrosequencing and bioinformatic analyses.

Artificial communities were assembled using 22 cyanobacterial strains from the BCCM/ULC Polar Cyanobacteria Collection. Library Art1 ('even') was prepared by pooling the DNA of all strains at equal concentrations, while in library Art2 ('staggered') DNA from nine strains accounted for 80% of the community. Six microbial mats from aquatic habitats of Continental Antarctica and the Sub-Antarctic were also analysed. DNA was extracted from the mats using the PowerSoil DNA Isolation Kit (MoBio) according to manufacturer instructions. Sample TM2 was also processed using a dedicated DNA extraction (Taton et al. 2003).

The cyanobacteria-specific primer set 359F and 781Ra/781Rb (Nübel et al. 1997) was used to amplify the V3-V4 variable region of the 16S rRNA gene from both the artificial and environmental samples. Sample RI2 was analysed as two technical replicates in order to assess the reproducibility of the method, which were carried out using the same DNA extract but independent PCR reactions and sequencing. Moreover, to assess potential biases arising from direct long primer amplification, samples RI2 and TM2 were also analysed using a modified two-step PCR protocol. Sequences were obtained using the 454 GS FLX+ Titanium platform.

Sequence data obtained from the artificial communities were used to assess the performance of five bioinformatic pipelines for quality control of reads, removal of chimeric sequences and OTU clustering. Pipelines (I) 'shhh.flows (450 flows)', (II) 'shhh.flows (360-720 flows)' and (III) 'Sliding Window (Q35, 50 bp)' were carried out using mothur according to Schloss et al. (2011). Pipelines (IV) 'fastq_maxee' and (V) 'fastq_truncqual' were carried out using UPARSE according to Edgar (2013).

From the 22 original sequences used to assemble the artificial communities, at least 21 were recovered at the end of each bioinformatic pipeline. However, observed relative abundances differed from the expected and also varied slightly between pipelines. Observed phylotype richness varied considerably between pipelines, ranging from 20 to 261 OTUs and from 16 to 155 OTUs in communities Art1 and Art2, respectively. The mothur-based protocols (pipelines I to III) generated a high number of additional erroneous OTUs (82-240 OTUs). On the other hand, the frequency of erroneous OTUs was much lower in the UPARSE-based datasets. Community structures observed in the technical replicates or using different DNA extraction protocols were statistically similar (Unifrac p-value < 0.05). Moreover, the use of barcoded primers did not influence community structure.

Our results show that, even with highly stringent quality controls, the presence of chimeric sequences significantly inflate phylotype richness estimates. Recovered microbial community structures were pipeline-dependent, with UPARSE being able to generate the most accurate community structure information. These findings also show the importance of assessing the performance of different bioinformatic pipelines using artificial communities, in order to efficiently assess the contribution of PCR and sequencing errors to the distortion of community structure estimates.

KEYWORDS: CYANOBACTERIA, 454 PYROSEQUENCING, BIOINFORMATICS

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Poster E-08

BASELINE DATA ON THE CYANOBACTERIAL DIVERSITY OF SVALBARD ASSESSED BY PYROSEQUENCING

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Cyanobacteria are common photosynthetic microorganisms in high latitude ecosystems where they generally form biofilms, crusts, and mats in aquatic and terrestrial biotopes (Vincent 2000). They appear to be adapted to the harsh conditions and tolerate freeze/thaw cycles, seasonnaly contrasted light availability, UV radiations, dessication and salinity. Cyanobacteria are important photosynthetic organisms in these ecosystems, due to their roles in soil aggregation, nitrogen fixation, carbon cycles, secondary metabolite production, among others. Furthermore, the presence of biogeographical patterns in microorganisms, as found in macroorganisms, is an ongoing debate. The existence of cyanobacterial taxa common to both Poles is currently discussed (Jungblut et al. 2010, Strunecky et al. 2010).

As Global Climate Change continues to increase, the structure and function of pioneering microorganisms are likely to change in an unpredictable manner. The overall aim of this study was to investigate and characterize cyanobacterial communities (using a Next Generation Sequencing methods) in previously unexplored High Arctic Regions around Svalbard, in addition to understanding their spatial patterns. In the present study, during the 2013 MicroFun expedition, we sampled 72 locations around Svalbard including very diverse biotopes such as glacial forefields, tundra soils, hot springs, soil crusts, microbial mats, wet walls, cryoconites, plankton in ponds and periphyton, in order to (1) assess the biodiversity of cyanobacteria around Svalbard, (2) verify the existence of biogeographical trends around the archipelago, and (3) compare these data with other polar (cold) areas, especially Antarctica. With this study, we aim to extend the baseline information of cyanobacterial diversity through an integrated and standardized analysis of the microbial diversity in the sampled environments.

We used a pyrosequencing approach targeting cyanobacteria-specific 16S rRNA gene sequences to deeply study the cyanobacterial communities. Hundreds of cyanobacterial OTUs at 97.5% similarity were found, indicating a previously uncharacterized enormous diversity of these microorganisms around the archipelago. OTUs matching species of *Arthronema, Coleofasciculus, Leptolynbya, Lyngbya, Microcoleus, Nostoc, Oscillatoria*, and *Phormidium* were most abundant in the different locations. Preliminary assessment indicates that there are largely distributed 'cold-adapted' OTUs as well as unique ones only found in Svalbard. When considering the ecological range, some taxa appear unique to a particular biotope, but others can be observed in different environments.

KEYWORDS: SVALBARD, CYANOBACTERIA, BIODIVERSITY, BIOGEOGRAPHY, PYROSEQUENCING

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Poster E-09

THE BCCM/ULC COLLECTION TO CONSERVE, DOCUMENT AND EXPLORE THE POLAR CYANOBACTERIAL DIVERSITY

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In Polar Regions, Cyanobacteria represent key primary producers and are the main drivers of the food webs in a wide range of aquatic to terrestrial habitats. For example, they form benthic microbial mats in lakes and soil crusts in terrestrial biotopes. They have adapted to their environment, and may present interesting features to survive freeze/thaw cycles, seasonally contrasted light intensities, high UV radiations, dessication and other environmental stresses.

The BCCM/ULC public collection funded by the Belgian Science Policy Office since 2011 aims to gather a representative portion of the polar cyanobacterial diversity with different ecological origins (limnetic microbial mats, soil crusts, cryoconites, endoliths, etc.). The collection is available for researchers to study the taxonomy, evolution, adaptations to extreme environmental conditions, and genomic make-up. It presently includes 200 cyanobacterial strains, with 123 being of polar origin (catalogue: http://bccm.belspo.be/catalogues/ulc-catalogue-search).

The morphological identification shows that the strains belong to the orders Synechococcales, Oscillatoriales, Pleurocapsales, Chroococcidiopsidales and Nostocales. The large diversity is also supported by the phylogenetic analyses based on the 16S rRNA sequences. This broad distribution makes the BCCM/ULC collection particularly interesting for phylogenomic studies. To this end, the sequencing of the complete genome of 16 selected strains is currently under way.

In addition, cyanobacteria produce a wide range of secondary metabolites (e.g. alkaloides, cyclic and linear peptides, polyketides) with different bioactive potential (e.g. antibiotic, antiviral, anticancer, cytotoxic, genotoxic). Bioassays have shown antifungal activities of the cell extracts from strains *Plectolyngbya hodgsonii* ULC009 and *Phormidium priestleyi* ULC026. The potential of the polar strains to produce cyanotoxins and other secondary metabolites is currently being studied by ELISA, LC-MS and the detection of genes involved in their production. Due to the geographic isolation and the strong environmental stressors of the habitat, the exploration of these metabolites in Antarctic cyanobacterial strains seems promising for biotechnology or biomedical applications (Biondi et al. 2008).

In summary, the BCCM/ULC public collection could serve as a Biological Resource Centre (OECD 2001) to conserve and document the biodiversity of polar cyanobacteria, as well as a repository for discovery of novel bioactive compounds.

KEYWORDS: POLAR CYANOBACTERIA, CULTURE COLLECTION, DIVERSITY, BIOACTIVITY

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Session F
Polar/alpine eukaryotic microorganisms

Keynote lecture KN-F

POST-MIOCENE DIVERGENCE OF POLAR DIATOM BIOMES

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Contemporary theory and concepts in macro-ecology consider both real-time processes such as dispersal and deterministic (selection) and stochastic (drift) changes in population densities, and long-term processes (e.g., speciation and the response to past climate and environmental perturbations) in order to explain the present-day distribution of floras and faunas. In microorganisms, the relative roles of contemporary and mid- to long-term processes in structuring their communities remain unclear. We studied the present-day lacustrine Arctic and Antarctic diatom biomes using a dataset containing records from >650 lakes and dovetailed this with Miocene and Pleistocene fossil assemblages to assess the role of long-term and real-time community assembly processes. We used the polar regions as a test case, because they share similar present-day environmental boundary conditions, while they differ in climate and tectonic histories and the configuration of their land masses. While connectivity is high in terrestrial habitats at the Northern high latitudes, ice-free terrain is highly isolated in the Antarctic Realm, constraining dispersal and movement of taxa in response to climate changes and ice sheet expansions and contractions. The extant diatom floras in comparable Arctic and Antarctic lakes are highly divergent. Only ~5.5% of the species occurred in both polar regions. The Arctic flora is relatively diverse, dominated by globally successful genera, and relatively homogeneous across different regions. In contrast, Antarctic communities are impoverished and imbalanced relative to their Arctic counterparts and possess a high level of endemism, the absence of particular genera and key functional groups such as planktonic taxa, an overrepresentation of terrestrial lineages, and clear bioregionalisation patterns. More in particular, the main biogeographic regions generally recognized in plants and animals (i.e., Sub-Antarctica, Maritime Antarctica, and Continental Antarctica) are also present in diatoms from the Antarctic Realm with a significant further subdivision into biogeographic provinces. Comparison of extant communities with fossilized assemblages revealed that before the Mid Miocene cooling event (c. 12 Ma), the Antarctic flora shared floristic affinities with both present-day polar diatom floras, and contained species belonging to genera which have been present in the Arctic for at least the past 2.5 Ma, as well as taxa currently restricted to the old Gondwanan continents of Southern South America and Australasia. Lake sediment cores from Antarctica spanning the penultimate glacial-interglacial cycle further revealed the survival of well-adapted taxa in refugia during the Last Glacial Maximum. This suggests that the observed divergence between the polar diatom floras occurred after full glacial conditions became established in Antarctica during the Mid Miocene and the subsequent Neogene and Pleistocene glacial-interglacial cycles. This likely led to the regional extinction and selective survival of diatom lineages resulting in an imbalanced flora. This, together with local speciation likely resulted in the high incidence of endemism and biogeographic provincialism in the Antarctic diatom flora. In the Arctic most lineages were apparently able to follow the waxing and waning of ice sheets and survived in refugia further to the South or cryptic refugia at Northern latitudes during glacial maxima. These orbitally-paced dispersal and recolonization events likely led to the homogenous Arctic diatom flora as also observed in macroscopic organisms today. We conclude that post-Miocene evolutionary trajectories and environmental history left a clear imprint on the present-day distribution and biodiversity of polar diatoms biomes.

KEYWORDS: DIATOMS, BIPOLAR DISTRIBUTION, EXTINCTION, SPECIATION, DISPERSAL LIMITATION, ENDEMISM, BIOGEOGRAPHY

Lecture F-01

BLACK YEASTS FROM GLACIERS TO SAUNAS – BIOLOGICAL ANSWER TO A CHANGING WORLD?

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Black yeasts are typical inhabitants of extreme natural environments, from salterns, phylloplane, rocks in semi-arid and desert environments, and glacial ice. Some of them show high prevalence in the human environment and are frequently isolated from saunas and steam bath facilities, in sink drains, and drinking water. Recently they have also been discovered in extreme domestic environments, such as dishwashers and washing machines. These findings atrracted a lot of public attention and raised many questions concerning the routes of entry, dissemination to the exterior environment, the natural reservoirs and the virulence of these fungi for the human host. This adaptability to conditions as different as those found in glaciers, saunas and dishwashers, requires an impressive phenotypic plasticity, which might in turn explain their success as emerging human pathogens, able of biotope switches.

This strategy of high adaptability and versatility of responses and tolerance to a wide variety (or even combinations) of different stressors is employed by the species that have been described as polyextremotolerant generalists and can be exemplified by species of the black yeast genera *Exophiala* and *Aureobasidium*.

Exophiala dermatitidis and *Aureobasidium pullulans* are not only isolated from anthropogenic habitats, which are characterized by rather high temperatures and oxidative stress, but – interestingly – also from glaciers in the Italian Alps or in Norway, in their meltwater, as well as in various the Arctic environments. These two species, as well as some other similar fungi, became important model organism for studies of versatile polyextremotolerant fungi by various systems biology methods including genomics, proteomics and transcriptomics. Initial research revealed some characteristics of their high ecological plasticity, including the “fine tuning” of the proteome and a wide array of extracellular enzymes present in the genome.

KEYWORDS: BLACK YEASTS, EXOPHIALA DERMATITIDIS, AUREOBASIDUM PULLULANS, GLACIERS, POLYEXTREMOTOLERANT FUNGI

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Lecture F-02

BIOGEOGRAPHIC ZONING OF AQUATIC MICROEUKRAYOTES IN THE ANTARCTIC REALM

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Increasing human activity and amplified climate change in the polar regions necessitate an improved understanding on how microbial terrestrial biodiversity is structured. A powerful approach involves the study of biogeographical patterns which are ideally suited to address key global change issues at immediately relevant spatial scales (i.e. continental to global). This is because these patterns yield important insights into the fate of biomes in response to future warming, such as range size expansions and contractions of taxa, and (regional) extinctions. To date, regional scale biodiversity and biogeographic patterns in limnetic eukaryotic organisms in the Antarctic biogeographic realm remains relatively poorly studied. We performed a large scale metagenomic survey of benthic communities of 28 sub-Antarctic (46-54°S) and 98 Antarctic (62-84°S) lakes along broad geographical, climatic and environmental gradients. Eukaryotic assemblages were taxonomically profiled with rbcL and 18S rRNA marker genes, using paired-end Illumina MiSeq sequencing. OTUs were assigned to the main taxonomic groups using the Wang classifier algorithm. Metazoa, Chlorophyta and Stramenopila, followed by Fungi, Ciliophora and Cercozoa accounted for the majority of the reads. Local OTU richness decreased with increasing latitude, corresponding to a six-fold decrease in average richness from 150 OTUs/sample (46° S) to 25 OTUs/sample (84°S). Moreover, Sub-Antarctic assemblages harboured more complex foodwebs, with arthropods, nemotods, rotifers, flatworms and annelids as main metazoan groups. Lakes on the continent, however, were characterised by fewer metazoan groups and a greater importance of microbial herbivores and secondary consumers, including a relative high diversity of ciliates and tardigrades. Using multivariate ordination techniques, distinct biogeograpic zones could be recognized in the distribution patterns of Stramenopiles, Ciliophora, Cercozoa, Chlorophytes and most Metazoa. This bioregionalisation is in agreement with the classical subdivision of the Antarctic Realm into Maritime Antarctica, Continental Antarctica and the Sub-Antarctic Islands generally observed in plants and animals. In contrast, Dinophyta and the Metazoan group Rotifera exhibited no clear biogeographic patterns. Variation partitioning analysis revealed that spatial variables that approximated large-scale regional contrasts in historical (e.g. deglaciation history, geological origin) and climatic factors (e.g. mean annual air temperature) significantly explained the largest portion of the observed variation in community structure, while differences in environmental variables were less important. In conclusion, our data suggest that climate history and geographical position have left a strong imprint on aquatic foodweb structure and taxonomic composition of benthic microbiomes in the Antarctic Realm.

KEYWORDS: ANTARCTICA, BIOGEOGRAPHY, MICROEUKARYOTES, LAKES, AMPLICON SEQUENCING, BIODIVERSITY

Lecture F-03

HUNTING FOR GREEN ALGAE AND CYANOBACTERIA IN SIBERIAN PERMAFROST

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The sporadic presence of green algae and cyanobacteria has been documented in permafrost sediments of lake-swamp and lake-alluvium origin (Vishnivetskaya 2009). Recently two metagenomes from permafrost samples of lake origin on river floodplain and the late Pleistocene Ice Complex collected in the Kolyma-Indigirka Lowland have been published (Krivushin et.al. 2015). In addition, a metagenome of the modern tundra cryosol from the same area is privately available on MG-RAST. We analyzed these metagenomes to track the abundance and distribution of photosynthetic organisms including green algae of the division Chlorophyta and cyanobacteria. Primers specific for most abundant algae groups were designed based on community analyses of metagenome data. The permafrost samples obtained in the Kolyma-Indigirka Lowland in northeast Siberia (69°299N, 156°599E) by slow rotary drilling without the use of any drilling solutions were used to start enrichment cultures on BG-11 medium at room temperature and constant low intensity white light illumination. The enrichments were incubated as long as four months, and enrichments with visible algae growth were further analyzed.

Based on the metagenome sequencing data the alpha-diversity was significantly higher in cryosol sample, however diversity did not differ much in the permafrost samples of similar age but different origin. The photosynthetic organisms including plants, green algae and cyanobacteria represented only <0.001% of total sequences. Among them the sequences affiliated with Streptophyta (86.4-91.5% of total sequences affiliated with photosynthetic organisms) were dominant, followed by Chlorophyta (5.1-7.6%), Cyanobacteria (3.8-4.9%), and Phaeophyceae (1.8-4.1%). There was no difference in abundance between Chlorophyta and Cyanobacteria in the late Pleistocene Ice Complex sediments, however samples of lake origin on river floodplain and tundra cryosol had Chlorophyta being more abundant in comparison to Cyanobacteria, which correspond to their distribution in modern tundra environment found in this region by others (Zenova et Shtina 1990). A total of 64 samples originated from river floodplain of 5 K years old, and lake-alluvium loams of 200 K, 600 K, 2 million years old were screened for presence of the photosynthetic microorganisms, which were found in 20 (31%) samples. Isolated algae were sub-cultured on BG-11 supplemented with 1.5% agar (Difco), single colonies were transferred to liquid BG-11 and incubated at room temperature and constant illumination for 2-3 weeks. DNA was isolated using FastDNA™ SPIN Kit (MP Biomedicals), and 18S rRNA was amplified and sequenced at the University of Tennessee Sequencing Facility. Green algae of the genus *Chlorella* were found in young and old permafrost samples. Presence of cyanobacteria was sporadic, however filamentous cyanobacteria related to *Oscillatoria* sp. were isolated from lake-alluvium loams of 200 K and 2 million years old. The genomic and metabolic features of isolated strains are being studied. It will provide insight into the long-term adaptation of photosynthetic organisms and their metabolic functions at constant low temperature and absence of light.

KEYWORDS: PERMAFROST, KOLYMA, METAGENOME, MICROALGAE, CYANOBACTERIA

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Poster F-01

THE BIODIVERSITY AND PLASTICITY OF SYMBIOTIC *Desmodesmus* SPP. (CHLOROPHYCEAE) FROM A SUBARCTIC SEA

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To maintain their homeostasis and the stability of the symbiosis, the microalgae forming, together with invertebrate hosts, multicomponent symbioses including assorted heterotrophic, phototrophic, and diazotrophic (cyano)bacteria (Gorelova et al. 2009, Gorelova et al. 2013) need versatile metabolic adjustments. This is especially true for species from a subarctic sea with extremely volatile environmental conditions e.g. Rugozerskaya Guba at Kandalaksha Bay of the White Sea. Accordingly, symbiotic microalgae from this locality should be a suitable model for study of metabolic plasticity and stress tolerance of autotrophs.

We studied the acclimation to nitrogen (N) starvation of subarctic symbiotic *Desmodesmus* representatives from the sponge *Halichondria panicea* (1Hp86E-2), trochophore larvae of the polychaete *Phyllodoce maculata* (1Pm66B), and the hydroids *Dynamena pumila* (3Dp86E-1), and *Coryne lovenii* (2Cl66E). Earlier we showed that the isolates differed in the epistructure morphology; type and number of the inclusions e.g. starch grains and oil bodies; fatty acid composition; in *Desmodesmus* sp. 1Hp86E-2 these differences were most pronounced. Molecular phylogenetic analysis showed that the isolates formed a monophyletic group with 1Hp86E-2 representing a distinct branch within this group (Gorelova et al. 2014a).

The microalgae were grown in N-replete or N-free BG-11 medium under continuous illumination of 110 µmol PAR photons m^{-2} s^{-1} and studied as described elsewhere (Gorelova et al. 2014b).

The lack of N induced, in a strain-dependent manner, a significant decline in the growth rate and a rearrangement of the cell organelles. Still most of the cells retained their ultrastructural integrity, including the cell epistructures. All strains featured a reduction of lamellae system of the chloroplast accompanied by a decline in chlorophyll and an increase in carotenoid-to-chlorophyll ratio and plastoglobuli abundance. However, the reorganization of thylakoids was strain-specific: in 2Cl66E and 3Dp86E-1, the thylakoid lumen expanded whereas in 1Hp86E-2 the thylakoid membranes become very thin (10.4±0.01 nm for the oppressed membranes). A lysis of the pyrenoid was observed in the 2Cl66E cells. All microalgae accumulated reserve lipids in cytoplasmic oil bodies and starch in chloroplast but the strains differed in the kinetics and level of lipid accumulation as well as in the major fatty acid (oleic and α-linolenic) ratio. The thickness of cell wall polysaccharide layer increased by up to 173% (in 1Hp86E-2).

The symbiotic *Desmodesmus* spp. differ in their N starvation tolerance, cell ultrastructural responses related with surplus carbon partitioning (between OB or CW) and N sparing (degradation of chlorophyll, thylakoid proteins and RuBisCo). Consequently, closely related microalgae from the same climatic zone but from different hosts may differ in their acclimation capabilities.

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KEYWORDS: ADAPTATION, NITROGEN DEFICIENT, SYMBIOTIC MICROALGAE, ULTRASTRUCTURE

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Poster F-02

DIATOMS FROM THE MARITIME ANTARCTIC REGION; EXTREME ENDEMISM IN ANTARCTICA.

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The terrestrial and freshwater aquatic habitats of the Antarctic region include Sub-Antarctic Islands, Maritime Peninsula and continent (Chown et Convey 2007), and differ significantly in climatic parameters and ecosystem types. In the past, Continental Antarctica has been described as biologically barren, cold desert with only a few species (Convey 2010). However, a variety of methods, including morphological and molecular techniques, have started to highlight that the Antarctic region is more diverse that has been assumed in the past for at least some groups.

Diatoms (Bacillariophyta) are one of the principal algal groups in the freshwater and terrestrial ecosystems of the Antarctic Region and are often used as indicators of environmental change as well as paleo-ecological and biogeographical studies. Until recently, most of the fresh-water diatom species were believed to have a cosmopolitan distribution, although recent studies show that a highly specific flora could be found in Maritime and sub-Antarctic Region. In the last 10 years, applying LM and SEM tools, and a fine-grained taxonomic approach, a large number of new *Luticola* taxa have been described from Antarctic region (Esposito et al. 2008, Kohler et al. 2015, Kopalová et al. 2009, Kopalová et al. 2011, Van de Vijver et al. 2001, Van de Vijver et al. 2006, Van de Vijver and Mataloni 2008; Van de Vijver et al. 2012b,. Zidarova et al. 2014), making the genus one of the most species-rich in the area.

We conducted a literature review based on a modern fine scale taxonomy to obtain a current assessment of distribution and endemic nature of species within the genus *Luticola*.

Studies on the genus *Luticola* from Antarctica show high levels of endemism in general and interesting biogeographic patterns. In total, 45 *Luticola* species have been found in the Antarctica region, of which 9 of the species are found in the sub-Antarctic region, and 7 of them are endemic to this region. In Maritime Antarctica, a total of 29 *Luticola* species have been found, and 24 of them are found only in that region (some of them presented in figure 2). Of the 14 species found on continental Antarctica, 9 are endemic. Of the total number of taxa encountered (45), only 1 is found outside the Antarctica region (*L. cohnii*), 1 is found in all three regions (*L. muticopsis*), and 3 are found in Maritime + Continental Antarctica (*L. austraoatlantica*, *L. gaussii* and *L. permuticopsis*); no species are found with the distribution of sub-Antarctic islands and Continental Antarctica to the exclusion of Maritime Antarctica. Thus, for the entire region the level of endemism is 98%, and lower but still substantial levels of endemism occur in the three regions. For the genus as a whole, nearly 20% of all species are endemic to Antarctica.

The diversity and levels of endemism seen in the genus *Luticola* are unmatched by any other known group of organism across a wide range of biodiversity in the Antarctic region. The pristine nature of Antarctica and its freshwater diatom flora will allow us to further explore what appears to be one of, if not the most, extreme cases of endemism ever described for life in Antarctica. Not only is the flora of *Luticola* species high in endemics in the sub-Antarctica region (44 of the 45 species currently described from the region are endemic to the, but of the total number of species in the genus (ca. 200; Levkov et al. 2012), 20% of them are endemic to Antarctica. There is only one cosmopolitan species known, further suggesting the pristine nature of the region, and avoiding the intermixing of cosmopolitan and endemic taxa. *This represents the highest degree of endemism at the level of genus in the Antarctic region for any other group of organisms on earth*.

Can we consider Maritime Antarctic region as a biogeographical hotspot or is it just a taxonomical artefact?

The present lecture discusses the diatom communities living in various freshwater habitats such as lakes, streams and seepage areas and mosses in relation to ecological factors determining their composition and discussing the high endemism found in the genus *Luticola*.

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Poster F-03

AN INTRIGUING SPEICIES OF *Eunotia* (BACILLARIOPHYTA) FROM GOUGH ISLAND (TRISTAN DA CUNHA ARCHIPELAGO)

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Gough Island (40°21' S, 9°53' W), is a small, remote, uninhabited island of a volcanic origin, positioned in the southern Atlantic Ocean, 350 km south of the Tristan da Cunha archipelago. Despite its very interesting geographical position, its oceanic origin, its extreme isolation and the number of interesting habitats, the non-marine diatom flora of Gough Island is only poorly known. Carter (1966) described a large number of new diatom species from the Tristan da Cunha Island group, reporting the presence of a very diverse diatom flora with a large number of new taxa, so far only rarely observed elsewhere. Recently, a new survey of the moss-inhabiting diatom flora of Gough Island started. Dominant genera in the investigated material include *Eunotia, Frustulia* and *Pinnularia*. Several taxa of these genera could not be identified using the currently available literature.
One species, originally described by Carter (1966) in the genus *Pseudoeunotia* as *P. linearis*, but most likely belonging to the genus *Eunotia* is of our particular interest. This species shows several morphological features that are unusual for the genus *Eunotia*, raising questions about its true taxonomic position. This poster presents and discusses its morphology based on detailed LM and SEM observations. More specifically, the overall valve shape of the valves, the presence of spines on the margins, the areola and stria density and structure, as well as the absence of helictoglossae are illustrated and discussed. Pictures are added from the original slide used by Carter in 1966. The transfer of the species to the genus Eunotia is briefly discussed and notes on its ecology are added.

KEYWORDS: GOUGH ISLAND, DIATOMS, EUNOTIA

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Poster F-04

REVISION OF THE GENUS *Nitzschia* IN THE MARITIME ANTARCTIC REGION

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The Maritime Antarctic Region comprises the Antarctic Peninsula and several islands and archipelagos in the southern Atlantic Ocean. Although the variation of freshwater habitats is rather limited, the Region presents a sufficient amount of opportunities for the diatom microflora to develop. For a long time, due to the force-fitting of most taxa into north-American and European names and taxonomic drift, the non-marine diatom flora inhabiting this Region, was considered to be composed of mainly cosmopolitan taxa. Recently, several species-rich genera have been revised resulting in the description of a large number of new taxa. Despite these taxonomic efforts, several genera, such as *Nitzschia* and *Gomphonema*, still awaited a thorough revision.

During the ongoing taxonomical revision of the limno-terrestiral diatom flora in Maritime Antarctic Region thirteen *Nitzschia* taxa were observed. Nine of them could not be identified using the currently available literature, were found.

The poster shows LM and SEM observations of six unknown *Nitzschia* taxa observed on Byers Peninsula (Livingston Island) and Clearwater Mesa and Ulu Peninsula (James Ross Island). Some of these taxa have frequently been reported in the past under cosmopolitan names such as *N. commutata* or *N. perminuta* but our taxonomic analyses revealed important morphological differences.

Although the genus *Nitzschia* is present worldwide, our analyses indicate that a large number of *Nitzschia* taxa show a restricted distribution within the Antarctic Region, pointing once more to a clear bioregionalism.

KEYWORDS: *Nitzschia*, MARITIME ANTARCTIC REGION, TAXONOMY, NEW SPECIES

Poster F-05

THE GENUS *Luticola* D.G.MANN (BACILLARIOPHYTA) FROM THE MCMURDO SOUND REGION, ANTARCTICA, WITH THE DESCRIPTION OF FOUR NEW SPECIES

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Species of the genus *Luticola* D.G.Mann in Round et al. 1990 are typical for terrestrial ecosystems in the Antarctic Region. The genus is characterized by uniseriate striae composed of rounded to transapically elongate areolae covered internally by perforated hymens, an isolated pore in the central area, a longitudinal canal positioned within the valve wall, and a simple filiform raphe with variable raphe endings (Round et al. 1990). A book volume for the genus was recently published by Levkov et al. (2013), which included 34 Antarctic Region taxa. A large number of new *Luticola* taxa have been described from Antarctica in recent years (Esposito et al. 2008, Kopalová et al. 2011, Van de Vijver et al. 2012, Zidarova et al. 2014), making the genus one of the most species-rich in the area.

The *Luticola* species of Maritime Antarctica and the sub-Antarctic Islands have been recently revised, showing that the genus *Luticola* is particularly widespread in the former region with more than 25 taxa compared to the sub-Antarctic islands where only 8 taxa were found. However, focused taxonomic work on the genus from the Antarctic continent was lacking, making a complete biogeographical analysis of the Antarctic Region not possible at present. The McMurdo Dry Valleys (MDVs) are adjacent to Cape Royds, and are home to the McMurdo Long-Term Ecological Research (MCMLTER) program, which has been monitoring Dry Valley streams and associated algal mat transects for over 20 years (mcmlter.org). Within this long-term research, *Luticola* species have been demonstrated to dominate Antarctic diatom communities, both in diversity and relative abundaces. In order to avoid erroneous conclusions that could be made when comparing biogeographical and ecological work from other regions (e.g. Kopalová et Van de Vijver 2013, Kopalová et al. 2013), a standardized Antarctic taxonomy was necessary and a reinvestigation into this flora using modern methods and literature is timely.

To promote compatibility among researchers and with other localities in the Antarctic Region, we analyzed samples for the genus *Luticola* taken by the MCMLTER program, which are stored at the Institute of Arctic and Alpine Research (INSTAAR) herbarium at the University of Colorado at Boulder, USA. Additionally, we resampled the lakes of Cape Royds over a century after West et West (1911) in order to evaluate their taxonomic designations, and compare this flora with the original material sampled by Shackelton's expedition stored at the British National History Museum in London, UK. All recovered species from the genus *Luticola* were compared using the most current literature, and are discussed based on detailed light and scanning electron microscopy observations. We here describe four new *Luticola* species, create one new combination, update synonyms to the most current accepted taxonomic unit, and compare all observed McMurdo Sound species with *Luticola* from the other parts of Antarctic Region (the sub- and Maritime Antarctic islands). A revision of the freshwater diatom genus *Luticola* from the McMurdo Sound Region, including the McMurdo Dry Valleys and Cape Royds, Antarctica, was made to contribute to a consistent flora for the entire Antarctic Region.

Detailed light and scanning electron microscopic observations, review of pertinent literature, and examination of historical and type material lead to the identification of 12 *Luticola* species. Four new species and one new combination are proposed, including *L. bradyi sp. nov.*, *L. spainiae*, *sp. nov.*, *L. macknightiae*, *sp. nov.*, *L. transantarctica*, *sp. nov.*, and *L. elegans*, *comb. nov. stat. nov*. Several of these taxa were previously identified as part of the *L. muticopsis* (Van Heurck) D.G.Mann complex; *Navicula muticopsis* f. *evoluta* W. & G.S. West, *L. muticopsis* f. *reducta* (W. & G.S. West) Spaulding, and *N. muticopsis* f. *capitata* Carlson, or mistaken for the similar *L. mutica* (Kützing) D.G.Mann and *L. cohnii* (Hilse) D.G.Mann. Morphological features of all new species were compared to the closest morphologically similar taxa, and their ecology and biogeography are discussed. All *Luticola* species considered here show restricted Antarctic distributions, and 8 of the 12 reported species are known only from the Antarctic continent.

Based on the results of this study and recent literature, our observations indicate the presence of 43 different *Luticola* taxa confirmed from the Antarctic Region. Following the application of a more fine-grained taxonomy, almost 98 % of all *Luticola* species within this grouping are restricted to the Antarctic Region, with a majority (53

%) confirmed only from the Maritime Antarctic. Furthermore, 21 % are known only from the Antarctic continent, and 14 % are typically present only in the sub-Antarctic region. The results also reveal that less than 10 % of the total number of observed species (*L. austroatlantica*, *L. permuticopsis*, *L. gaussii*, and *L. muticopsis*) is shared between the Antarctic continent and Maritime Antarctic Region (MA/CA, not counting *L. cohnii*). There is no evidence for a species only shared between the sub-Antarctic and the Antarctic continent without being also present in the Maritime Antarctic Region, and there is no species that is shared exclusively between the sub- and Maritime Antarctic. Only one species, *L. muticopsis*, is present across the entire Antarctic Region. These results are in strong contrast to previous reports of the biogeographical features of the Antarctic diatom flora, where a majority of the species was considered to be cosmopolitan.

KEY WORDS: BIOGEOGRAPHY, CAPE ROYDS, DIATOM TAXONOMY, DRY VALLEYS, MICROBIAL MATS, ROSS ISLAND.

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Poster F-06

EXPOSITION OF VARIOUS STRESS CONDITIONS ON FILAMENTOUS GREEN ALGA *Klebsormidium* (STREPTOPHYTA) ISOLATED FROM BOTH POLAR REGIONS AND SLOVAKIA

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The filamentous green alga *Klebsormidium* (Klebsormidiales, Streptophyta) is one of the most widespread green algal genera found around the world and an interesting candidate for investigations of various stress scenarios. Strains isolated from distinct geographical regions (Antarctic – South Shetlands, King George Island, Arctic – Ellesmere Island, Svalbard, Central Europe – Slovakia) were experimentally exposed to various stress factors. The level of resistance was evaluated by vitality tests. Selected strains were generally resistant to freezing. Lyophilization was the most harmful regime for studied strains. Only three Arctic strains, one strain from Slovakia and one strain from Antarctica indicated high level of resistance to this stressor. On the other hand, two Antarctic strains indicated low level of resistance to environmental stresses.

ACKNOWLEDGEMENT: This work was supported by the Slovak Research and Development Agency APVV (Project no. SK-CZ-2013-0019) and projects by the Ministry of Education, Youth and Sports of the Czech Republic, nos. 306010-7AMB14SK135 and CzechPolar LM 2010009.

KEYWORDS: *Klebsormidium*, GREEN ALGAE, ANTARCTIC, ARCTIC, SLOVAKIA, STRESS CONDITIONS, VITALITY

Poster F-07

NEW RECORD OF CRYOSESTON ON OLYMPUS MT., GREECE

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Coloured snow was mentioned long since by Aristotle in his book „The history of animals“. He mentioned: „… worms are found in long-lying snow; and snow of this description gets reddish in colour, …." (Thompson, translation). Ice warm *Mesenchytraeus*, however, is not cause of red snow, but the snow algae are food for the animal. The ice warm has been found in N.America, till now and it is strange where Aristotle could see the coloured snow settled with ice worms.
Olympus Mt., Greece is prospective place for snow algae, the highest peak rises to 2,919 metres, and snow is persisting through May. We sampled there this May and we found there: *Chloromonas nivalis* (dominate in lower altitudes), *Cryocystis nivalis, Koliella nivalis*, also fungi *Chinaster nivalis* and *Selenotila nivalis.* In higher altitudes we expect also *Chlamydomonas nivalis*, and *Koliella viretii*.

ACKNOWLEDGEMENT: The research was also supported by projects LM2010009 CzechPolar - Czech polar stations: Construction and logistic expenses (MŠMT), as priority topic of scientific cooperation between ASCR and BAS 2014 – 2016 No. 23 Study of biotechnological potential of extremophilic and extremotolerant algae and cyanobacteria, and a long-term research development project no. RVO 67985939 (IB)

KEYWORDS: CRYOSESTON, OLYMPUS MT., GREECE

REFERENCES:
http://classics.mit.edu/Aristotle/history_anim.5.v.html on May 17, 2015.

Poster F-08

UNIQUE DIVERSITY OF MARINE MICROBIAL EUKARYOTES IN THE HIGH ARCTIC (SVALBARD)

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In Arctic waters, microbial eukaryotes mitigate the carbon and energy needed by other trophic levels, since cyanobacteria are virtually absent in the Arctic marine environment. These microbes are always the primary photosynthetic functional group (Li et al. 2009). Understanding microbial diversity in marine systems, the evolutionary processes that give rise to them, and the way their communities create and maintain ecosystem function and biogeochemical cycles are vital goals in biology. Microbial eukaryotes are morphologically, phylogenetically, and functionally diverse organisms living as single cells, though many form colonies of a few to several cells. They are a polyphyletic group, since the common origin of all groups goes back to LECA. These microbes are abundant and almost ubiquitously distributed on Earth, and the presence of biogeographical patterns in their distribution is an ongoing debate.

The Arctic Ocean is surrounded by land, and some consider it a large estuary (McClelland et al. 2012). Through the Bering Strait, it is connected to the Pacific Ocean and to the Atlantic via the Fram and Davis Straits and the Barents Sea. The Gulf Stream system transports warm and salty waters from the Atlantic into the Arctic Ocean, and one of its main branches is the West Spitsbergen Current. This current influences the marine system off of western and northern Svalbard, which also receives glacial meltwaters in the different fjords. With this study, we aim to describe the common and unique diversity of microbial eukaryotes, which we have found through several ongoing projects studying the diversity and function of these organisms.

We used clone libraries, pyrosequencing, and Illumina sequencing targeting the V4 region of the 18S rDNA to deeply study the diversity of microbial eukaryotes in Spitsbergen waters. Hundreds of OTUs at 97-98% have been found, with several novel clades and tips, especially in the Alveolates. These studies indicate that diversity is mostly related to season and watermass with the existence of a previously uncharacterized diversity of these organisms in waters off Spitsbergen. Preliminary analyses agree with previous studies (see Lovejoy 2014) in which there are largely distributed 'cold-adapted' OTUs.

KEYWORDS: SVALBARD, MICROBIAL EUKARYOTES, NEXT GENERATION SEQUENCING, BIODIVERSITY, BIOGEOGRAPHY

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Poster F-09

EPIPHYTIC DIATOM COMMUNITIES FROM TERRA NOVA BAY AND CAPE EVANS (ROSS SEA) - A SYNTHESIS

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INTRODUCTION

There is growing evidence that a large proportion of the Antarctic herbivorous fauna feed mainly on benthic diatoms, which constitute a high quality and readily available food source. However, although epiphytic diatom communities clearly must play a particularly important role in the functioning of Antarctic shallow water ecosystems, many aspects of their ecology, taxonomy, distribution and biodiversity remain understudied and poorly understood. Typically epiphtytic diatom species are rarely reported in paleoenvironmental studies although they are often found in Antarctic marine sediments. There is currently a pressing need for taxonomic and ecological characterization of polar diatoms for application in both paleoenvironmental and monitoring studies. Nevertheless, for the generally unknown and unidentified Antarctic benthic species, environmental roles and significance cannot yet be established and, thus, the utility of benthic diatoms in paleoecological studies is limited. Therefore, detailed investigation of the contemporary distribution and diversity of Ross Sea epiphytic diatom communities and the factors controlling these is important to the understanding and proper interpretation of modern and past variations in the benthic diatom flora of this region.

This work builds on the results of a recent survey of marine epiphytic diatoms from the Ross Sea (Majewska and De Stefano 2014; Majewska et al. 2013a, Majewska et al. 2013b, Majewska et al. 2014) aiming at providing a thorough description of Ross Sea epiphytic diatoms, their distributions, and the factors that may affect them.

MATERIALS AND METHODS

Macroalgal material was obtained during 11 summer Antarctic expeditions to Terra Nova Bay and Cape Evans (Ross Sea) in the seasons 1989/90 – 2011/12. Thalli of three common macroalgal species (*Iridaea cordata*, *Phyllophora antarctica*, *Plocamium cartilagineum*) were collected by SCUBA divers from regularly used sampling locations: Tethys Bay, Molo, Faraglione, Adélie Cove, Cape Russell, and Cape Evans.

Diatoms were identified and enumerated on a surface area of ca. 1-2 mm^2 of each of the 166 collected macroalgal individuals at magnifications ranging between 400x to 60000x using a scanning electron microscope. In addition, diatom communities associated with epiphytic sessile fauna were also analysed.

RESULTS AND DISCUSSION

A total of 109 diatom species (44 genera) was found during the survey. Three species, *Cocconeis fasiolata*, *Fragilariopsis nana* and *Navicula perminuta* occurred in all samples. Diatom communities found during the three summer months at various depths and sampling stations differed significantly in terms of species composition, growth form structure and abundances. Their densities ranged from 21 to more than 8000 cells mm^{-2}, and were significantly higher on the surface of epiphytic micro-fauna than on any of the macroalgal species examined. Generally, host organisms characterized by higher morphological heterogeneity (sessile microfauna, ramified *Plocamium*) supported richer diatom communities than those with more uniform surfaces (*Iridaea*). Difference between epiphytic communities associated with different macroalgae was reflected better in species composition than in growth form structure. The latter changed significantly with season: adnate diatoms dominated clearly in December, whereas motile forms were the most abundant group in January and February. The highest contributions of planktonic species to total diatom number were observed in January, which was associated with sea ice breaking-up and melting. The total diatom abundance increased gradually from December to February. Although ice cover strongly influenced epiphytic diatom community composition, it neither inhibited their growth nor limited significantly their abundance.

KEYWORDS: EPIPHYTIC DIATOMS, GROWTH FORM, HOST SPECIFICITY, ICE INFLUENCE, MACROALGAE

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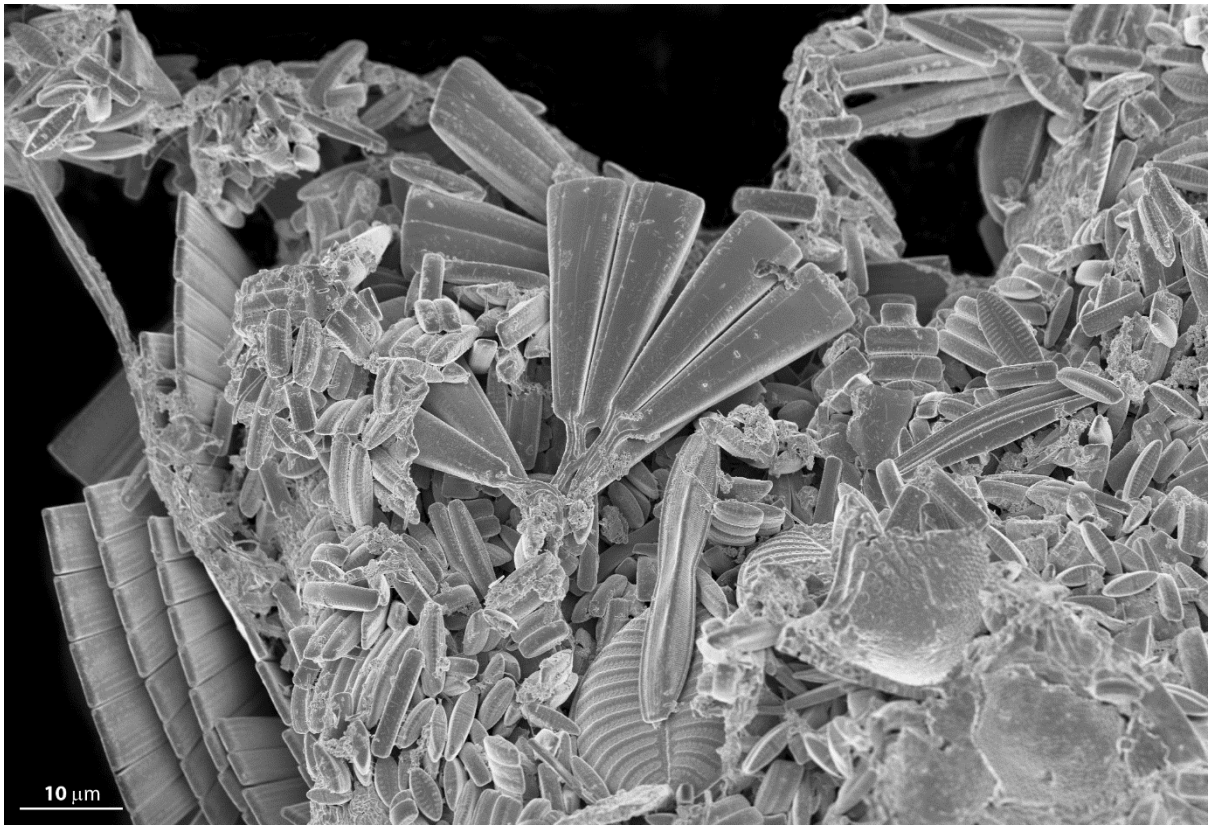

Fig.1. Epiphytic diatoms on *Plocamium cartilagineum*.

Poster F-10

TEMPORAL AND SPATIAL VARIATIONS IN PIGMENT COMPOSITIONS OF SNOW ALGAE IN Mt. TATEYAMA IN TOYAMA PREFECTURE, JAPAN

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Snow algae are photosynthetic microorganisms inhabiting on alpine and polar snow fields. When they bloom, they can change color of snow to red or green since they have various pigments in their cells. Variation in snow color may be associated with environmental conditions and/or taxa of the algae. However, detailed information is not known. In this study, we analyzed pigment compositions, microscopic cell morphology and abundance, and 18S rRNA gene of algal snow collected in the melting season of 2014 on Mt. Tateyama in Toyama prefecture, Japan. We aim to understand the relationship among taxa, life cycles, and pigments of algae.

Absorption spectrums of extracts from the colored snow showed that there were four absorption maximums in absorption spectrums. Each absorption maximums may correspond to pigments contained in the algae, including Chlorophyll *a*, Astaxanthin and unknown Carotenoid. Absorption spectrums varied among the samples, and that could be classified into 4 types: Type A (with maximums of Chlorophyll *a* and Astaxanthin), B (with maximums of Chlorophyll *a* and unknown Carotenoid), C (with maximums of Chlorophyll *a* only), and D (without any maximum). Microscopy of the samples revealed that the samples of A and B types contained snow algae of different color and structure: red sphere cells in Type A, and orange sphere, yellow sphere, green oblong cells in Type B. Analyses of the 18S rRNA gene identified 15 OTUs of algal gene in the samples. The samples of Type A and B contained distinctive OTUs of the algae, respectively, suggesting that the difference of algal pigments between Type A and B is not due to pigment compositions in same algal taxon, but to those of different algal taxa. Analysis of seasonal changes revealed that pigment compositions changed from Type A to Type B at the same location during the study period, suggesting that algal species composition on the snow surface change with time. The results also showed that the colored snow of Type A, B, and C appeared on several locations in Tateyama mountains from June to August. Variations in algal species and pigment compositions among time and locations may be attributed to life cycles and the dispersal of algae.

KEYWORDS: TATEYAMA, SNOW ALGAE, CHLOROPHYLL *a*, ASTAXANTHIN, CAROTENOID

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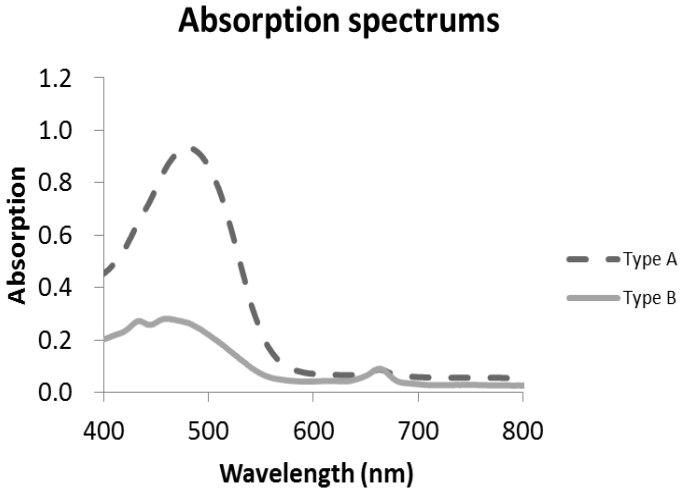

Poster F-11

IDENTITY AND ECOPHYSIOLOGY OF COCCOID GREEN ALGA DOMINATING IN ICE-COVERED LAKES ON JAMES ROSS ISLAND (NE ANTARCTIC PENINSULA)

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During the limnological survey of lakes in the northern deglaciated part of James Ross Island (NE Antarctic Peninsula), we examined the origin, geomorphological position, physico-chemical and biological characteristics in a representative set of 29 lakes. A variety of lake types could be distinguished ranging from stable shallow lakes that originated several thousand years ago to deep cirque and kettle lakes with the maximum expected age of approximately a century or rather some decades. Deep lakes are located at higher altitude (> 200 m a.s.l.) and their key feature is the development of extensive ice cover, which persists at least partly during summer. At the time of the sampling, the ice was up to 2 m thick and had a characteristic candle-like structure (up to 30 cm long) causing important light extinction. The lake water was characterised by low temperature (<1 °C) and an isothermal vertical profile during the period of measurement. Whereas high conductivity and DOC were characteristic for shallow coastal lakes, there were high soluble reactive phosphorus and nitrate concentrations in deep lakes (Nedbalová et al. 2013).

The phytoplankton was dominated by a small coccoid species that was tentatively determined according to its morphology as a green alga *Monoraphidium* sp. Its abundances reached ~10^5 cells.ml^{-1} resulting in relatively high chlorophyll *a* concentrations (3-7 µg.l^{-1}). In contrast to massive autotrophic mats in the littoral zone of shallow lakes, these assemblages were only poorly developed in deep lakes. The lake biota was further characterized by the common occurrence of a calanoid copepod (*Boeckella poppei*) that apparently used the phytoplankton as main food source.

Two laboratory strains of *Monoraphidium* sp. from a cirque and from a kettle lake were studied. Their morphology was described using optical and TEM microscopy and phylogenetic analysis based on 18S rDNA was performed. The taxonomic position of the isolates within the family Selenastraceae (class Chlorophyceae) was confirmed and *Monoraphidium dybowskii* was identified as a closely related taxon. The members of the Selenastraceae are very common in freshwater habitats and exhibit high morphological diversity. Available data suggest the status of some genera including the needle-shaped *Monoraphidium* and morphologically similar *Ankistrodesmus* have to be reconsidered and *Monoraphidium dybowskii* clade should be included in a new genus in the future (Krienitz & Bock 2012). Even though the presence of this group was reported from Antarctic lacustrine habitats, the dominance of a non-motile alga in ice-covered lakes with very limited mixing is rather surprising.

The growth optima of the two strains were tested using crossed gradients of temperature and light. The cultures were unable to grow at temperatures above 20 °C suggesting their psychrophilic nature. Low light requirements reflect the conditions in the original habitat. The strains cultivated at 3 °C were found to possess high levels of polyunsaturated fatty acids (PUFAs), which is a feature linked to successful survival in permanently cold habitats (Morgan-Kiss et al. 2006).

To conclude, the green coccoid alga *Monoraphidium* sp. represents an ecologically important species in ice-covered lakes on James Ross Island, NE Antarctic Peninsula. Its ecophysiology is well adapted to this extreme, but stable habitat, making this species a prospective subject for further studies.

KEYWORDS: PHYTOPLANTON, CHLOROPHYTA, ECOPHYSIOLOGY, LAKE, JAMES ROSS ISLAND

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Poster F-12

HIDDEN DIVERSITY: MULTIPLE ARCTIC AND ANTARCTIC LINEAGES IN THE COSMOPOLITAN DIATOM *Pinnularia borealis*

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Pinnularia borealis Ehrenb. is a common diatom (Bacillariophyta) found on all continents. Although it has been observed in Antarctic waters with low nutrient and mineral content, *P. borealis* is mainly confined to (semi)terrestrial habitats such as seepages, moss vegetation and moist to dry soils. In the past, a large number of morphological forms have been described as subspecies, varieties or formas, but many of these show considerable morphological overlap resulting in an uncertain taxonomic status. Recently, detailed morphological analysis of several forms occuring in Amsterdam Island (sub-Antarctic) and Livingston Island (Maritime Antarctic) resulted in the description of inter alia *P. sylviae* Van de Vijver and *P. quesadae* Van de Vijver & Zidarova as species new to science.

In parallel, a molecular phylogenetic approach was taken to tackle the species diversity, evolutionary history and biogeography of this enigmatic diatom. During field campaigns on various localities, including alpine, Arctic and Antarctic regions, samples were taken from soils, moss, seepages and the littoral zones of freshwater ponds and lakes. When *P. borealis* was present, cells were isolated, brought into culture and harvested for DNA and morphology analysis. In a first effort, samples were collected from Schirmacher Oasis (Continental Antarctic), Canada, Chile, Mongolia, Czech Republic, France and Belgium. Molecular phylogenies based on the plastid gene *rbc*L and the nuclear LSU rDNA revealed the presence of 8 lineages in *P. borealis*, including a distinct continental Antarctic lineage (Souffreau et al. 2013). A molecular clock estimates the origin of *P. borealis* at 30-47 million years ago and the age of the continental Antarctic lineage at 8 million years. Since then, the addition of 31 strains from Marion Island (sub-Antarctic), Vega Island (Maritime Antarctic), Spitsbergen (High Arctic) and Belgium resulted in the discovery of no less than 8 additional lineages and the co-existence of multiple lineages within a single region. Within individual lineages, different haplotypes were detected from different locations, suggesting the presence of an intra-lineage phylogeographic structure. Morphological differentiation between the different lineages seems incomplete, as several taxa cannot be reliably separated using light microscopy or scanning elecktron microscopy, while others do correspond to newly described varieties or species in the complex. Very recently, new strains were obtained from the Pyrenees (Spain), Norway, James Ross Island and the South Shetland Islands (Maritime Antarctic), and morphological and genetic data will be assessed in the coming months.

All together, the results already indicate a high degree of hidden species diversity in *P. borealis*, and hint at a high regional species diversity in the Maritime Antarctic and the presence of regional endemics in the (sub)Antarctic. Future in-depth analyses of this complex in polar regions based on additional isolates and environmental sequencing approaches, will allow addressing key questions concerning its species diversity, evolutionary history, biogeography and ecological differentiation in Arctic and Antarctic regions.

KEYWORDS: BACILLARIOPHYTA, *Pinnularia borealis*, BIOGEOGRAPHY, MOLECULAR PHYLOGENY, POLAR REGIONS

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Poster F-13

DIVERSITY AND DISPERSAL CAPACITIES OF ARCTIC TERRESTRIAL ALGAE

David Ryšánek, Josef Elster, Pavel Škaloud

Distribution of microalgae represents a highly discussed topic in modern microbial research. Two opposite hypotheses have been proposed: the ubiquity model emphasizing the cosmopolitan distribution of protists; and the moderate endemicity model, which admit the existence of endemic species with limited distribution. In our study, we examined the diversity and distribution of the filamentous green algal genus *Klebsormidium* in polar regions. This genus is very common and diverse in temperate zones, but data about its occurence in polar regions are very scarce. In total, we isolated 32 *Klebsormidium* strains in both Arctic and Antarctic regions. Genetic investigation of these strains revealed their affiliation to the 7 distinct genotypes. In comparison with temperate regions, the observed diversity in polar regions is rather low and clearly opposite to the distribution of the genus *Xanthonema* forming a dominant component of arctic algal assemblages. The majority of arctic strains were inferred within the cosmopolitan clade B. To investigate in detail the dispersal patterns of terrestrial protists, all available *Klebsormidium* strains belonging to the clade B were subjected to the population study. We designed three new primer combinations to sequence highly variable spacer regions in the chloroplast genome. The results showed a significant, recent dispersal of the algal genotypes across the continents and climatic zones.

Session G
Biotechnology at low temperatures

Keynote lecture KN-G

BIOTECHNOLOGICAL SIGNIFICANCE OF MICROORGANISMS IN LOW TEMPERATURE ENVIRONMENTS

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Low temperature environments on Earth have been successfully colonized by psychrophilic (cold-adapted) microorganisms. Cold adaptation includes a complex range of structural and functional adaptations at the level of all cellular constituents, and these adaptations render cold-adapted microorganisms particularly useful for biotechnological applications. This presentation will give an overview of the exploitation of cold-adapted microorganisms as a source of cold-active enzymes and biopolymers and of their benefits for food microbiology, bioremediation and biocontrol.

KEYWORDS: BIOTECHNOLOGY, APPLICATION, BACTERIA, YEAST

Lecture G-01

BIOPROSPECTING OF Hg PROCESSING MICRO-ORGANISMS FROM SOUTH SHETLAND ISLAND, ANTARCTICA

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Several studies warn about the contamination of the Antarctic environment. Among many, causes high heavy metal concentrations in Antarctic sediments and bioaccumulation in the marine food network are highlighted by many researchers. The main objectives of this work were to determine mercury levels in sediment and Antarctic seabirds feathers and evaluate if microorganism isolated from these samples have the potential for mercury bioremediation.

Samples were processed by culture and molecular techniques including 454 pyrosequencing. The samples were collected in Dee, Barrientos and Greenwich Islands located in South Shetland Island in the Antarctic Peninsula during summer 2013 and 2014. Feather samples were processed both years and including Skúa (*Chataracta lonnbergi*) and gentoo *(Pygocelys papua)* and chinstrap penguins (*Pygocelys. antarctica*). Sediment samples were processed only 2013 and one Barrientos sample was carried out pyrosequencing and results were analyzed using the Quantitative Insights Into Microbial Ecology QIIME software to metagenomic.

Samples of sediments and feathers were enriched in a medium with mercury 18mg/L and 3mg/L respectively in different media Peptone Water, Luria Bertani and Potato Dextrose Broth incubating at 10°C; approximately every 15 days total mercury was determined via atomic absorption spectrophotometry using a Direct Mercury Analyzer (DMA-80). Microorganisms isolated were identified followed by PCR amplification and sequencing of the ribosomal ITS1, 5.8s, and ITS2 DNA region and the highest identity percentages (98% identity or above) from BLAST.

Microorganisms isolated correspond to yeast and microfungi species of genus in sediment species of *Glaciozyma*, *Mrakia (blollopis), Pseudeurotium*.; in feathers *Meyerozyma (guilliermondii), Cryptococcus (flavescens, terrestris)*, *Rhodotorula (mucilaginosa)*, *Pseudogymnoascus*, *Penicillium* (*adametzoides, brevicompactum)*; being *Debaryomyces hansenii* present in all types of samples.

Pyrosequencing results showed OTUs assigned to 625 genera of bacteria the most representative were: Leptolyngbya (34.88%); Phormidium (13.92%); Arthrobacter (0.96%); Sporosarcina; Candidatus Solibacter (0.48%), and , 8% were not assigned to any group.

Samples Reduction of mercury levels by microbial communities after 15 days of incubation in sediment was around 95%, in penguins 80%, and Skúa between 44-80%.

Experiments using individual isolates are ongoing, the results show the potential of these microbial communities for mercury biorremediation and for future research to determine genes responsible for Hg accumulating/processing capacities.

KEYWORDS: ANTARTICA, MERCURY, FEATHERS, SEDIMENT, BIORREMEDIATON

Lecture G-02

DEVELOPMENT OF PHOTOBIOREACTORS FOR LOW-TEMPERATURE ENVIRONMENT

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In polar regions microalgae and cyanobacteria mass cultivation has not developed yet. Polar summer offers continuous light and relatively stable temperature conditions for 3-6 months (October-February), which may be suitable for mass cultivation of some low temperature adapted microalgae and cyanobacteria species. However, to cope with the harsh climatic conditions of polar regions (particularly low temperature) it is necessary to develop a photobioreactor geometry which can take advantage of the low solar altitude. Recently, a novel photobioreactor design (1350 L) has been devised and constructed by us for the cultivation outdoors of microalgae. The photobioreactor is composed of 20 vertical parallel flat panels with adjustable distance each other. This reactor features the following main important characteristics. 1) high surface to volume ratio; 2) high ratio between illuminated and ground area; 3) optimal mixing achieved with an optimal pump design; 4) easy to start since culture inoculation can be carried out step by step (e.g., a panel at a time); 5) full control of culture parameters (temperature, pH, oxygen). This PBR design is proposed for both high value compounds and energy production (hydrogen) in low temperature regions. Wherever possible, the bioreactor should be developed and integrated with existing polar infrastructures and nutrient/energy sources. Most of the experiments should be carried out using indigenous strains. However, in order to assess the performance of this novel PBR design, we have carried out preliminary experiments with the cyanobacterium *Synechocystis* PCC 6803 grown under optimal temperature conditions. The daily biomass yield reached with this cyanobacterium reached 25 g/m2/day. One of the serious problems that arose during the cultivation of *Synechocystis* outdoors was the susceptibility of the cells to predation by various type of protozoa and other class of algae. Practical methods for preventing contamination in large-scale PBR have been successfully tested.

Lecture G-03

NAPHTHALENE-DEGRADING BACTERIA ASSOCIATED WITH TERRICOLOUS LICHENS IN ICELAND

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INTRODUCTION

Terricolous lichens form a prominent part of the vegetation in the Arctic and sub-Arctic, often forming large, dense mats. Lichens and their associated bacteria can thus be expected to play a relatively important role in various ecosystem services in these fragile environments, including degradation of environmental pollutants. These systems thus comprise a promising, but thus far surprisingly little-tapped venue for bioprospecting for cold-active bioremediation bacteria. We have found that the associated bacteriome of many Arctic and sub-Arctic lichen species harbours efficient scavenges of organic and inorganic nutrients, including efficient biodegraders belonging to the classes Alphaproteobacteria, Betaproteobacteria, Actinobacteria and others, many of which are easily culturable (Sigurbjörnsdóttir et al. 2014, 2015).

METHODS AND MATERIALS

Lichens were aseptically sampled in various locations in northern and central Iceland. After repeated washing with sterile Butterfield's buffer (Bb), lichen thalli were crushed in a sterilized mortar, serially diluted in Bb and plated onto a variety of media, including commercial media such as R2A, Nutrient Agar, etc., and specialized media such as various lichen extract media, differential media, etc. Plates were incubated at 5, 15 or 25°C for up to several months, and representatives of all observed colony morpholotypes streaked for purification. A total of 703 purified and re-culturable isolates were suspended in 28% glycerol, frozen and stored at -70°C. Isolates were identified by sequencing of partial 16S rRNA gene amplicons using standard methods. Isolates were screened for naphthalene oxidation via a chromogenic fast-blue-mediated detection of naphthol. Biosurfactant production was tested by a simple drop-collapse assay.

RESULTS

More than 10% of the lichen-associated isolates tested were positive for naphthalene oxidation at 15°C. Most of the strains were psychrotrophic, being able to produce significant growth at 5°C. Most naphthalene-oxidizing strains were also able to produce biosurfactants. Among naphthalene-oxidizing taxa were Actinobacteria (*Dietzia* spp.), Alphaproteobacteria (*Sphingomonas* and *Polymorphobacter* spp.), Betaproteobacteria (*Burkholderia* spp.), Bacilli (*Bacillus* and *Paenibacillus* spp.) and Sphingobacteriia (*Pedobacter* and *Mucilaginibacter* spp.).

DISCUSSION

The Arctic terricolous lichen-associated bacteriome is an excellent source of cold-active potential bioremediation bacteria, yielding a taxonomically diverse, easily culturable collection of naphthalene degraders that may find use in the protection of the fragile Arctic environment in times of rapidly increasing anthropogenic impact.

KEYWORDS: LICHEN-ASSOCIATED BACTERIA, BIOPROSPECTING, BIOREMEDIATION

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Poster G-01

BIOSYNTHESIS OF GOLD NANOPARTICLES BY A CRYOTOLERANT CYANOBACTERIUM ISOLATED FROM SCARISOARA ICE CAVE (ROMANIA)

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Biotechnology of cold-loving microorganisms is an expanding scientific field (Feller et Margesin,2012; Mair et al. 2013). In this paper we report gold nanoparticles biosynthesis by a cryotolerant unicellular cyanobacterium isolated from Scarisoara Ice Cave (Romania) in comparison with the results mesophilic *Synechocystis* PCC 6803 strain. Special attention is focused on the followings:

i) gold nanoparticule biosynthesis by metabolically active cyanobacteria kept either in dark and in light (both types of photosystems being active), at different temperatures (5°, 15° or 25° C), each temperature having different physiological signification for these two strains;

ii) gold nanoparticles were extracted from intact cells by physical and chemical methods in order to obtain either naked GNP or covered GNP;

iii) the shape and size of gold nanoparticles synthesized in dark or in light by intact cold adapted cyanobacterium and by mesophilic strain, *Synechocystis* PCC 6803 kept at different temperatures were determined to check the effect-if any- of either temperature or light regime on these two parameters;

iv) The isolated gold nanoparticles were also analyzed with respect to their fluorescence spectra and the original data were discussed in correlation with their shape and size;

v) The ability of these living, metabolically active cyanobacteria to reduce gold ion to elemental gold which further self aggregate to produce gold nanoparticles is discussed in the context of their energetic metabolism with special concern for respiratory and photosynthetic electron transport; the aim is to add new insights into the biological signification of gold nanoparticles biosynthesis by cyanobacteria;

vi) The bio(nano)technological applicative potential of the obtained GNPs is currently under investigation, as well as the potential of cold loving cyanobacteria for other biotechnological topics (e.g. lipids production as raw material for biodiesel).

KEYWORDS: CYANOBACTERIA, CRYOTOLERANT,GOLD NANOPARTICLES SYNTHESIS

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Feller, G., Margesin, R. (2012): Polar microorganisms and biotechnology. In: Miller, R., Whyte, L.G.: Polar Microbiology: Life in a Deep freeze. Washington: ASM Press, pp. 166 – 180

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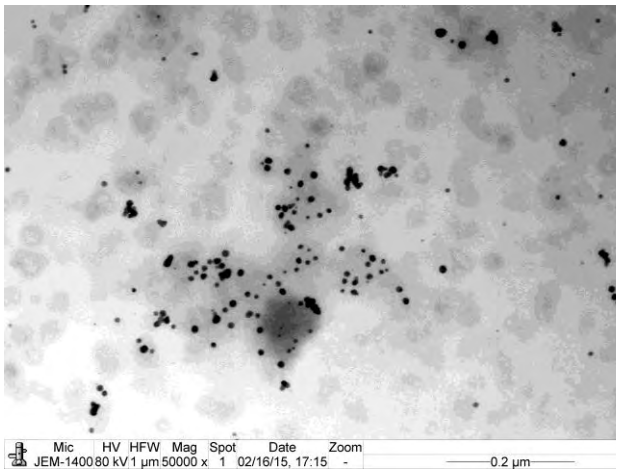

Fig.1. GNP synthesized in light by the cryotolerant cyanobacterium-

Poster G-02

BIOPROSPECTING PSYCHROTROPHIC SPHINGOMONADS FOR HYDROCARBON DEGRADATION

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INTRODUCTION

Sphingomonads are aerobic, heterotrophic Alphaproteobacteria characterized by the presence of glycosphingolipids in their outer cell membranes and the absence of lipopolysaccharides. Sphingomonads from cold environments are potentially of great value as cold-active bioremediators and may also be of interest for several industrial applications due to their relative ease of culturing and their rather wide spectrum of utilizable carbon sources, with many species being capable of degrading compounds normally considered recalcitrant to biotic degradation. We therefore sampled several cold habitats, including glacial meltwater, glacial moraines, tundra soils, arid soils, riverbeds, various lichens, and more, and isolated sphingomonads. Thirty-nine sphingomonad isolates were investigated in terms of their ability to degrade petrochemicals such as naphthalene and hexane.

MATERIALS AND METHODS

Samples (water, soil, lichens) were collected aseptically in various locations in northern and central Iceland. Water samples were plated directly. After repeated washing with sterile Butterfield's buffer (Bb), soil or lichen thalli were crushed in a sterilized mortar, serially diluted in Bb and plated onto a variety of media, including commercial media such as R2A, Nutrient Agar, etc., and specialized media such as various lichen extract media, differential media, etc. Plates were incubated at 5, 15 or 25°C for up to several months, and representatives of all observed colony morphotypes streaked for purification. A total of 2,819 purified and re-culturable isolates were suspended in 28% glycerol, frozen and stored at -70°C. Isolates were identified by sequencing of partial 16S rRNA gene amplicons using standard methods. Thirty-nine sphingomonad isolates were screened for naphthalene oxidation via a chromogenic fast-blue-mediated detection of naphthol. Degradation of biopolymers was tested on plates supplemented with azo cross-linked substrates. Biosurfactant production was tested by a simple drop-collapse assay. Ten selected strains were whole genome-sequenced by Illumina MiSeq sequencing of Nextera XT-prepared libraries. Assembly and BLAST-based annotation was performed using the CLC Genomic Workbench.

RESULTS

Thirty-nine isolates were assigned by partial 16S rDNA sequencing to the family Sphingomonadaceae. Several of these are considered likely to comprise novel species, based on their relatively low level of similarity to their closest relative in GenBank. Several of the strains tested positive for hydrocarbon degradation at low temperatures and/or display other cold-active oxidative or biodegradative capabilities, such as glycanase and esterase activities. Production of biosurfactants was also observed for several strains. Preliminary screening of the as-yet unannotated genome sequencing data indicates the presence of several oxygenase genes and other genes thought important for petrochemical degradation.

DISCUSSION

Spingomonads from cold environments in northern and central Iceland hold promise as efficient, easily cultured and manipulated, cold-active bioremediators of petrochemicals.

KEYWORDS: SPHINGOMONADACEAE, BIOPROSPECTING, BIOREMEDIATION

REFERENCES:

Sigurbjörnsdóttir, M. A., Heiðmarsson, S., Jónsdóttir, A. R., Vilhelmsson, O. (2014): Novel bacteria associated with Arctic seashore lichens have potential roles in nutrient scavenging. Canadian Journal of Microbiology 60: 307-317. doi: 10.1139/cjm-2013-0888.

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Poster G-03

POLYCHLORINATED BIPHENYL DEGRADING BACTERIA FROM THE KONGFJORDEN (SVALVARD ISLANDS, ARCTIC NORWAY)

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INTRODUCTION

Polychlorinated biphenyls (PCBs) were produced between 1929 and late 1970s and used in several industrial applications, due to their chemical and physical characteristics. They show bioaccumulation and biomagnification behaviours, thus posing a serious risk to the environment. Several pathways have been suggested for PCB contamination in Polar Regions, such as long-range transport by air and water, local contamination due to improper disposal practices, riverine inputs and local accumulation due to biotic activities. Despite their recalcitrant nature, PCBs can be biotransformed by different microbial species inhabiting both contaminated and uncontaminated sites. In this context, the present study was aimed at investigating the occurrence of cold-adapted PCB-oxidizing bacteria in an Arctic fjord.

METHODS

Enrichment cultures at 4°C on biphenyl (BP) were carried out using seawater and sediment samples that were collected along the Kongsfjorden (Svalvard Islands, Arctic Norway) in summer 2009. Aliquots of each enrichment were plated on a mineral salt medium in the presence of BP and used for bacterial isolation. Bacterial growth on PCBs was tested in a mineral salt medium that was added with Aroclor 1242 (100 ppm in dichloromethane) as sole carbon and energy source (final concentration 0.1 %, v/v). Aroclor 1242 is a mixture of PCB congeners (ranging from dichloro- to hexachlorobiphenyls) made of twelve carbon atoms in the biphenyl molecule and containing 42% chlorine by weight. The cultures were incubated at 4 C° for 3 weeks on a rotary shaker operated at 100 rpm. The ability to use PCBs as growth substrates was evaluated according to the degree of turbidity or the appearance of cellular flocs in the test tubes. Uninoculated medium was incubated in parallel as a negative control. Bacterial isolates growing of PCBs were identified by the 16S rRNA sequencing, and screened for the presence of the catabolic gene *bph*A involved in PCB degradation.

RESULTS

A total of 254 strains (179 and 75 strains from seawater and sediment samples, respectively) were obtained from enrichment cultures. Among them, 50 isolates that were able to grow in the presence of Aroclor 1242 as unique carbon source were identified. The 16S rRNA gene sequencing revealed that PCB-oxidizing bacteria mainly belonged to the *Gammaproteobacteria*, *Actinobacteria* and CF group of *Bacteroidetes*, in addition to few members of the *Firmicutes*. Among them, 18 isolates (mainly *Pseudomonas* spp.) harbored the gene fragment.

DISCUSSION

This work highlights the presence of cold-adapted bacteria able to aerobically degrade polychlorinated biphenyls in the Arctic marine environment. Results showed that the 19.7% of isolated strains were able to grow in the presence of PCBs. This percentage appears greater than those previously reported for bacteria from polar sites. The phylogenetic affiliation of PCB-oxidizing bacteria showed that the *Gammaproteobacteria* and *Actinobacteria* were the dominant phyla both in seawater and sediment. The CFB group of *Bacteroidetes* was more represented in sediment that in seawater, while the *Firmicutes* were found only in sediment samples. The analysis of the functional gene *bph*A confirmed the ability to degrade PCBs in selected strains. Further analyses will be carried out to establish their biodegradative potential by gas-chromatographic analyses.

KEYWORDS: BACTERIA, BIODEGRADATION, PCBs, FUCTIONAL GENE.

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Poster G-04

TOLERANCE TO HEAVY METALS AND POLYCHLORINATED BIPHENYL BIODEGRADATION POTENTIAL BY ARCTIC BACTERIA FROM CONTINENTAL NORWAY

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INTRODUCTION

Low temperature is responsible for reducing both metabolic and growth rates of organisms, thus promoting high concentrations of contaminants in the biota and accumulation throughout the food chain. Heavy metals (HMs) and polychlorinated biphenyls (PCBs) can have a negative effect on all forms of life. Once in the environment, these problematic contaminants cannot be easily dispersed. Their fate is strictly linked to bacteria which represent the first step in the transfer of toxic compounds to higher trophic levels. In this context, the present work was aimed at analysing the response of bacteria from Arctic sediment and water to HMs and PCBs, in order to provide further insights toward this topic in cold-systems.

METHODS AND MATERIALS

Sediment and water samples were collected from the Pasvik River and Varanger Fjord area (Arctic Norway). For the isolation of PCB-degrading bacteria, enrichment cultures (ECs) in Bushnell Haas medium (BH) added with biphenyl (BP) were performed with sediment samples. BP-utilizing bacteria were isolated by spreading aliquots of ECs on BH plates in presence of BP, and incubation at 4°C for 30 days. Isolates were screened for growth on Aroclor 1242 at 4 and 15°C. Positive strains were taxonomically identified by 16S rRNA gene sequencing, and additional analyses were performed to detect the *bph*A gene. HM-tolerant bacteria were obtained by directly spreading sample aliquots on solidified media amended with different concentrations (50 to 5000ppm) of HM salts. HM tolerant colonies were isolated from agar plates and further analyzed for multi-tolerance.

RESULTS

A total of 218 strains were isolated from BP-ECs. Among them, 31 grew in presence of Aroclor 1242. They belonged to the *Gammaproteobacteria* (82%), CF group of *Bacteroidetes* (9%), *Betaproteobacteria* (5%) and *Actinobacteria* (4%) within the genera *Pseudomonas* (10 strains), *Arthrobacter* (one strain), *Pusillimonas* (one strain), and *Algoriphagus* (one strain). The *bph*A was detected in ten strains belonging to the genera *Pseudomonas* and *Halomonas*, followed by *Arthrobacter* and *Pusillimonas*.

With regard to HM tolerance of bacterial communities from water, copper (Cu) was better tolerated (generally up to 500ppm) than other metals, which were generally tolerated at the minimum concentration tested, with the exception of isolates from stations 2-9 and 2-15 (up to 500ppm of zinc, Zn), and 2-16 (up to 1000ppm of Zn). Tolerance to all HMs, including mercury (Hg), was observed only for bacteria isolated from the station 2-19 (both in water and sediment). With regard to bacterial isolates from sediment, all HMs were generally tolerated up to 50 ppm. As for water, Cu was better tolerated than other metals, especially at stations 2-8, 2-17 and 2-19. In the presence of Zn and cadmium (Cd) bacterial growth was observed in four and two stations, respectively. No growth occurred at stations 2-15 and 2-16 in the presence of tested HMs. A total of 134 strains were isolated. Among them, 36 were multi-tolerant with 10 and 7 isolates that tolerated four and three HMs tested in this study, respectively. The remaining 19 isolates tolerated only two metals.

DISCUSSION

The 15.1% of strains were able to use Aroclor 1242 as the sole carbon source at low temperature, suggesting the PCB biodegradation potentiality of benthic bacterial communities in cold systems. *Pseudomonas* and *Arthrobacter* members have been previously reported as able to utilize PCBs, whereas no reports exist on such capability by the other genera mentioned above. Overall, tolerance to the HMs assayed in this study was in the order of Cu>Zn>Cd>Hg. Multi-tolerant isolates generally grew up to 1000ppm of Cu and Zn, and up to 100ppm of Cd and Hg.

Overall, results highlighted the important role of bacteria as autodepuration agents, and the biotechnological potential of psychrotolerant bacteria.

KEYWORDS: HEAVY METALS, POLYCHLORINATED BIPHENYLS, ARCTIC, AROCLOR, BPHA

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Poster G-05

BIOPROSPECTING OF ANTARCTIC MICROORGANISMS AND THEIR EXTREMOPHILES ENZYMES APPLIED IN THE FOOD INDUSTRY (AMYLASE)

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Bioprospecting of extremophilic microorganisms is continuously growing in the Antarctic continent. The study of Antarctic microorganisms has significantly grown in the last years mainly to obtaining novel low-temperature active biomolecules with industrial application in spite of in the food industry is not has been evaluated in depth. Antarctic sediment is the ideal candidate for bioprospecting. In past studies about bioprospecting indicates a relatively high diversity of microorganism present in Antarctic soil that contributes significantly to the hydrolysis of the major organic constituents and carbon cycling. The purpose of this study was to obtain psychrophilic enzymes from Antarctic's microorganisms and their application of it in the food industry. Apply the amylase in a specific step in process and determinate the enzymatic affectivity in flour dough. The Isolation and identification, enzymatic bioprospecting and, the production and evaluation of the enzymatic extract were the steps we follow to obtain the efficient microorganism and their enzymatic extract

The soil samples were taken in the Ecuadorian scientific station Pedro Vicente Maldonado in Antarctica. Around 137 bacteria isolates was obtained from soil samples and was tested by +/- enzymatic activity. Mineral agar containing starch, pectin, or lactose as carbon source was used for bioprospecting of cold-adapted amylases, pectinase, or lactases respectively. The bacteria positive to amylase activity were individually evaluated to establish the efficiency in substrate degradation in low temperature. Efficient bacteria were identification by sequencing part of the 16S region of bacterial isolates. The identified isolates were determined the optimal conditions of temperature and pH to production enzymes measured by protein production in mineral broth starch. The bacterial suspention was centrifuged to separate the bacteria and lyophilized to concentrate the extracellular amylase. Lyophilized supernatant was evaluated at 8 temperatures and 6 pHs to determine the optimal conditions using Baranyi and Rosso models. To the evaluation of amylase activity, the lyophilized extract was tested in dough at temperatures of 7°C, 20° at 40°C and compared with pure alpha amylase as control. Rheological parameter was established using a rheometer Kinexus Pro to get the modulus of elasticity (ΔG') and determine if there was a change in the elasticity of the dough incubated in low temperature

As a result, more than 74 isolates showed the presence of at least one of the enzymes of interest. The sequentiation of the isolates reveal most of them belonging to the genus *Pseudomonas, Arthrobacter, Rhodococcus, Achromobacter* and *Pantoea.* The most predominant genus in the samples identified is *Pseudomonas* with 69% of the total followed by *Arthrobacter* with 19% and *Rhodococcus* with 8%. The isolate CIBE-27 was selected to enzymatic production whose optimal temperature is 20°C and the pH around 6.5. The estimated protein concentration of the lyophilized supernatant indicate that for each milligram of lyophilized contain 0.1202 mg of protein. The flour dough was mixed with 0.5% protein concentration of lyophilized and tested in 7°C and 40°C. The CIBE-27 strain showed a lower value (ΔG') at 7°C to compare with pure alpha amylase which was inactive at this temperature. This result indicate that the CIBE-27 enzyme change the elasticity of the dough in low temperature.

Although the optimal range of the enzyme is 20 °C, is able to degrade starch at 7ºC. Psychrophilic microorganisms are considered those growing in temperature ranges from 0 ° C to 25 °C (2). Based in this quote CIBE-27 strain can be considered one on them. Results show the potential of this Antarctic enzyme in the food industry. DNA fragmentation and cloned into competent E. coli to increase the rate of production of the enzyme is the next step in the investigations.

KEYWORDS: BIOPROSPECTING, EXTREMOPHILIC, MICROORGANISMS, AMYLASE, *Arthrobacter*

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Session H
Astrobiology of icy worlds

Keynote lecture KN-H

POTENTIAL BIOSPHERES IN THE ICY WORLDS IN OUR SOLAR SYSTEM

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The challenge in astrobiology and planetary research in the near future is to realize space missions to study the habitability of Mars and the icy moons of the Jovian and Saturnian system. Mars is an interesting object to search for habitable environments and for fossilized life because of its past water driven wet history. On the other hand the Jovian moon Europa and Saturnian moon Enceladus are promising candidates, where liquid water oceans beneath the surface are expected. These oceans can be habitable environments and it would be good to search there for present life. Some examples on potential biospheres will be given, which might be able to live in such kind of icy environments.

KEYWORDS: ICY MOONS; MARS; BIOSPHERE; ASTROBIOLOGY

Lecture H-01

MICROBIOLOGY OF THE SUBGLACIAL LAKE VOSTOK: FIRST RESULTS WITH BOREHOLE-FROZEN LAKE WATER AND PROSPECTS

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The objective was to search for microbial life in the subglacial Lake Vostok (buried beneath 4-km thick East Antarctic ice sheet) by studying the accretion ice (naturally slowly frozen lake water) as well uppermost water layer entered the borehole upon lake entry (February 5, 2012) and then shortly got frozen within. The latest samples included the drillbit water frozen on a drill bit upon lake enter along with re-drilled borehole-frozen water ice.

The comprehensive analyses (constrained by Ancient DNA research criteria) showed that the accretion ice in general contains the very low microbial biomass. The only ice containing mica-clay inclusions (type I) allowed the recovery of few bacterial phylotypes all passing numerous contaminant controls. They included well-known chemolithoautotrophic thermophile *Hydrogenophilus thermoluteolus* (β-*Proteobacteria*), actinobacterium related to *Ilumatobacter fluminis* (95% similarity) along with unidentified unclassified bacterium AF532061 (92% similarity with closest relatives). In contrast, the deeper accretion ice (type II) with no sediments present gave no reliable signals.

As for the first lake water samples all they proved to be contaminated with drill fluid. The drillbit water was heavily polluted with drill fluid (at ratio 1:1) while borehole-frozen water samples were rather cleaner but still contained numerous micro-droplets of drill fluid. The cell concentrations measured by flow cytofluorometry showed 167 cells per ml in the drillbit water sample and 5.5 - 38 cells per ml in borehole-frozen samples.

DNA analyses came up with total 49 bacterial phylotypes discovered by sequencing of different regions of 16S rRNA genes. Of them only 2 phylotypes successfully passed all contamination criteria. The 1st remaining phylotype w123-10 proved to be hitherto-unknown type of bacterium showing less than 86% similarity with known taxa. Its phylogenetic assignment to bacterial divisions was also unsuccessful except it showed reliable clustering with the above mentioned unidentified bacterium detected in accretion ice. The 2nd phylotype is still dubious in terms of contamination. It showed 93% similarity with *Janthinobacterium sp* of *Oxalobacteraceae* (*Beta-Proteobacteria*) – well-known 'water-loving' bacteria. No archaea were detected in lake water frozen samples.

Thus, the unidentified unclassified bacterial phylotype w123-10 along with another one (AF532061) might represent ingenious cell populations in the subglacial Lake Vostok. The proof may come with farther analyses of cleanly collected lake water.

KEYWORDS: ANTARCTICA, SUBGLACIAL LAKE VOSTOK, BOREHOLE-FROZEN WATER, EXTREMOPHILES, UNKNOWN BACTERIA

Lecture H-02

BIOMEX EXPERIMENT: SURVIVAL, ULTRASTRUCTURAL AND MOLECULAR DAMAGE IN THE CRYPTOENDOLITHIC ANTARCTIC FUNGUS *Cryomyces antarcticus* EXPOSED TO SPACE AND SIMULATED MARS-LIKE CONDITIONS

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The search for traces of extinct or extant life in extraterrestrial environment or meteorites is one of the main goals for astrobiologists; due to their ability to withstand stressing conditions, extremophiles are perfect candidates for astrobiological studies. In this contest, the BIOMEX project aims to test the stability of biomolecules and cell components under space and Mars-like conditions, besides investigating survival capability of microorganisms (de Vera et al. 2012). The experiment has just been launched in space and is now exposed to space on the EXPOSE-R2 payload of the European Space Agency, outside the International Space Station (ISS) for 1.5 years. Along with a number of extremophilic microorganisms, the Antarctic cryptoendolithic black fungus *Cryomyces antarcticus* CCFEE 515 (Selbmann et al. 2005, Onofri et al. 2012) has been included in the experiment. Before launch, colonies grown on Lunar and Martian rock analogues were dried and exposed to different stresses in ground based experiments, including vacuum, or simulated Martian atmosphere, polychromatic UV, to test survival, ultrastructural and molecular damage. Our results on Scientific Verification Tests (SVT) reported that the fungus survived space simulated conditions, showing only slight ultrastructural and molecular damages.

KEYWORDS: GROUND BASED EXPERIMENTS, BLACK FUNGI, SURVIVAL, SPACE SIMULATION

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Lecture H-03

Methanosarcina soligelidi SMA-21 – AN ARCHAEAL CANDIDATE FOR LIFE ON MARS

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Terrestrial permafrost is of particular interest in the scope of astrobiological research as an analogue for extraterrestrial permafrost habitats, which is a common phenomenon in our solar system. Permafrost is characterised by extreme environmental conditions, such as sub-zero temperatures, aridity, and long-lasting levels of back-ground radiation as a result of an accumulation over geological time scales. Despite these harsh conditions, terrestrial permafrost is colonized by high numbers of chemoorganotrophic bacteria as well as microbes such as methanogenic archaea (Frank-Fahle et al. 2014). Because of the specific adaptations of methanogens to early Earth conditions (e.g. no oxygen, no or less organic compounds) and their phylogenetic origin, they are considered as one of the most probable model organisms for life in extraterrestrial permafrost such as on Mars. The aim of this study was to analyse the survival potential and metabolic activity of the new species *Methanosarcina soligelidi* SMA-21 (Wagner et al. 2013), which was isolated from a Siberian permafrost environment, when exposed to single and multiple simulated Martian environmental conditions.

For this purpose, *Methanosarcina soligelidi*, other methanogens from Siberian permafrost and reference organisms from non-permafrost habitats were subjected to long-term desiccation (400 days), UV (> 200 nm) and ionizing radiation, and ultra-low subfreezing temperature (-80ºC). Moreover, the influence of three different Mars regolith analogs (JSC Mars-1A, phylosilicatic and sulfatic MRAs) on the metabolic activity and growth of the exposed strains was analysed.

The results showed survival and methane production in all methanogenic strains under simulated martian environmental conditions, even if the different strains respond differently to the tested stress parameters. The experiments showed that methanogenic archaea are capable of producing methane when incubated on a water-saturated sedimentary matrix of regolith lacking additional nutrients (Schirmack et al. 2015). Survival of methanogens under these conditions was analysed by performing a long-term desiccation experiment in the presence of regolith analogs. All tested strains of methanogens survived the desiccation period as it was determined through reincubation on fresh medium and via quantitative PCR following propidium monoazide treatment to identify viable cells. The best results were achieved in presence of the phyllosilicate-rich Mars regolith analog. After exposure to subfreezing temperatures, Siberian permafrost strains had a faster metabolic recovery compared to non-permafrost methanogens. On the other hand, non-permafrost strains remained intact membranes to a greater extent after incubation at ultra-low temperatures than those from permafrost. Furthermore, exposure of *Methanosarcina soligelidi* to solar UV and ionizing radiation showed an unexpected high radiation resistance indicated by F_{10} (UV-C) and D_{10} (X-rays) values of *M. soligelidi*, which are comparable to values for the well-known highly radioresistant species *Deinococcus radiodurans*.

The survival of long-term desiccation, subfreezing temperatures, radiation and the ability of active metabolism on water-saturated Mars regolith analogs strengthens the possibility of methanogenic archaea, particularly *Methanosarcina soligelidi* SMA-21, or physiologically similar organisms to exist in environmental niches on Mars. Recently the whole genome sequence of *Methanosarcina soligelidi* SMA-21 was retreived and may provide further inside into the survival strategies of this organism (Alawi et al. 2015).

KEYWORDS: METHANOGENIC ARCHAEA, MARS REGOLITH ANALOGS, LONG-TERM DESICCATION, RADIATION RESISTANSE, PERMAFROST

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Poster H-03

ADVANCES IN LASER-INDUCED FLUORESCENCE EMISSION TECHNOLOGY (L.I.F.E.) AND PRELIMINARY MICROBIAL DATA FROM AN ANTARCTIC GLACIER

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The nutrient and water availability in supraglacial environments changes in response to global warming and the decrease in albedo, mainly caused by black carbon deposits, derived from incomplete combustion of fossil fuels, biomass and wildfires (Menon et al. 2010). As a consequence, microbial communities in supraglacial environments change in several ways (Lutz et al. 2014). Studies that focus on changes of carbon fluxes in glaciated environments were rarely conducted until now. In this study we are going to characterize microbial communities from Anuchin Glacier in Antarctica for the first time, using traditional sampling and laboratory analysis. Additionally, we further develop and test a novel tool (L.I.F.E.) for the detection and quantification of porphyrin biomarker molecules.
Our study site is located in the Otto-von-Gruber-Gebirge of central Dronning Maud Land, 90 kilometers inland from the Schirmacher Oasis, Antarctica. The Anuchin glacier flows into the largest perennially ice covered surface lake in East Antarctica (Lake Untersee) which was discovered by pilots during a German Antarctic expedition in 1939. Sampling took place during the Tawani Antarctic Expeditions in 2013 and 2014. In 2015, additional samples will be collected. Non-cryoconite surface ice samples along the middle moraine, including two parallel transects (outside the middle moraine) were collected with a motor-driven Kovacs ice corer and kept frozen in sterile whirlpacs. In total, 54 shallow ice cores were obtained. Additionally, 48 cryoconite holes were sampled with a Kovacs ice corer. The diameter and depth of the cryoconite holes were recorded. Cryoconite hole samples have been collected from the glacial surface and from Lake Untersee lake ice.
Standard laboratory analyses will be performed on all samples. The methods include the assessment of bacterial abundance (DAPI staining, epifluorescence microscopy), autotrophic productivity (infrared gas measurements for CO_2), heterotrophic productivity ([^{3}H] leucine incorporation) and NGS sequencing. Additional analyses for cryoconite samples include the measurement of granule sizes and radio carbon dating.
The standard methodology to detect microbial life as described before implies severe manipulation of the ecosystem. The sensitivity of many psychrophiles to even moderate changes in temperature results in a falsification of *in-situ* conditions. Further, the remoteness and difficult accessibility of cryospheric field sites lead to data sets with a low spatial end temporal resolution. Hence, data up-scaling is not reliable with common methods. Additionally, ice algae play a crucial role in supraglacial carbon flux interactions but in response missing methods for *in-situ* quantification, the supraglacial distribution could not be assessed yet on a large scale in high resolution.
A novel tool (L.I.F.E.) was built for the *in-situ* detection and quantification of photo pigments in supraglacial environments. L.I.F.E. occurs when matter absorbs a fraction of an incident laser beam and emits a longer wavelength (lower energy) photon. This technology is arguably the single most sensitive active photonic probe of biomolecular intracellular and extracellular targets that does not require sample preparation, sample destruction, or consumable resources other than power. The first prototype was tested in the Arctic, Antarctic, the Alps and in the Maroccan desert (Groemer et al. 2014). Here we present preliminary microbial data from the Anuchin glacier and advances in L.I.F.E. technology, its future applicability and importance for supraglacial studies in context with global change.

KEYWORDS: ICE, LASER-INDUCED FLUORESCENCE EMISSION (L.I.F.E.), NON-INVASIVE, PORPHYRIN, ANTARCTICA

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