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SHORT COMMUNICATIONS

Microbial Communities of Water Column of Lake Radok, East Antarctica, Dominated by Abundant Actinobacterium "*Candidatus* Planktophila limnetica"[1]

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The ice-free regions of the Antarctic continent (Antarctic oases [1]), have attracted the special attention of researchers from the very first studies, as regions more likely to harbor life. A prominent feature of their landscape is the presence of numerous different lakes. One such Lake is Lake Radok, located in the Amery Oasis (a so-called "mountain" oasis [2]) in East Antarctica.

Antarctic lakes are unique ecosystems which are characterized by low-temperature and, mainly, oligotrophic conditions supporting rather simple truncated food webs [3]. Studying the biodiversity and community structure of such lakes affords a great opportunity to understand the mechanisms of adaptation and evolution of microorganisms, as well as in the search for new species [4] which may have biotechnological application.

Lake Radok is covered with ice, up to 3 m in thickness through the year. However, during the summer season, the lake surface is partially open, forming both polynyas and shore ice. The water catchment area averages 20.1 km^2, the length is about 10 km, the width – 2.9 km [6] and the maximum depth about 367.5 m [5].

The lake is fed by Battye (Priozerny) Glacier meltwater, which also provides a floating ice tongue, penetrating the lake up to 2 km. Lake Radok is connected to Lake Biver (located 6 m below the level of Lake Radok) by the Mezhozernaya river, that proceeds it in Pagodroma Gorge. Seasonal drainage of extra water by this river during active ice and snow melt keeps the water level in Lake Radok constant [5].

The main ions (the total mineralization) in the upper horizon are 133 mg/l while in the bottom layer—about 100 mg/l. The water temperature changes slightly from 0.8°C at the surface to 1°C at depth. The water pH and dissolved oxygen content throughout the water column are 7.8 and 10.6 mg/l [5], respectively [6]. The nitrate concentration [7] and sulfate concentration [6] in the upper layer are 0.7 and 12.0 mg/l, although at the bottom—these become 0.8 and 10.1 mg/l, respectively.

The objective of this research was to study the microbial content and diversity of the Lake Radok water column using bacterial 16S rRNA gene sequencing for various regions of the gene. Here we report the results of only the dominant phylotypes (3 clones and more) recovered for the uppermost and bottommost layers of the water column.

Two water samples were collected by the Kemmerer sampler (volume 1l) at the deepest point of Lake Radok in February 2005 during the 50th Russian Antarctic Expedition (R1 sample—depth of 1.3 m, right beneath the floating ice sheet; R367 depth of 367 m, nearly at the bottom). The water samples were transported frozen to Grenoble, France. Microbial cell counts were obtained using flow cytometry using SYBR Green-I and a BD FACSAria device.

Due to an expected low biomass, samples were concentrated (1600–3000 times) using CENTRICON Plus-70 columns (5000 Da membrane filter) (Millipore, United States). Cells were mechanically disrupted (FastPrep, MP Biomedicals, United States) and genomic DNA (gDNA) extracted using the PowerSoil DNA isolation kit (MoBio Labs, United States).

The water concentration and gDNA extraction procedures were carried out inside a clean room (class 10 000) on a laminar flow bench (class 100) at the Laboratory of Glaciology and Geophysics of Environment (LGGE, Grenoble, France). To amplify bacterial 16S rRNA genes, three pairs of (highly degenerate) universal PCR primers were used (GM338Fnewto & Com2-905Rm [8, 9], 515mF & 1397mR and w001mCFm & 1492R) targeting the variable areas v3–v5, v4–v8 and the full gene respectively.

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The distribution of dominant phylotypes in the upper- and bottommost horizons of the Lake Radok water column as revealed by sequencing of three different 16S rDNA gene regions

Closest taxon in GenBank (accession No.)	Similarity, %	% of clones in two horizons^{-1}	Horizon 1.3-m clone libraries, %			Horizon 367-m clone libraries, %		
			v3-v5	v4-v8	full-gene	v3-v5	v4-v8	full-gene
Prokaryotes								
Actinobacteria								
Candidatus Planktophila limnetica (FJ428831.1)								
acI-AVI clade	96.9	**27 + 23**	**27***			**23**		
acI-AIV clade	96.2	**17 + 11**	**17**				**11**	
unidentified clade of acI-A subgroup	97.4	**0 + 25**				**25**		
Mycobacterium vanbaalenii (NR_029293)	98.8	**26 + 16**	**10**	**26**		**16**	**16**	
Ilumatobacter fluminis (AB360343)	93.9	**0 + 5**				5		
α-proteobacteria								
Roseomonas frigidaquae (EU290160)	97.4	**0 + 8**				**4**	**8**	
Rhodovarius lipocyclicus (NR_025629)	95.2	**0 + 14**					**14**	
β-proteobacteria								
Curvibacter putative (FN543107)	99.5	**13 + 0**		**13**				
δ-proteobacteria								
Desulfomicrobium hypogeium (AF132738)	83	**0 + 4**				**4****		
Bacteroidetes								
Chitinophaga sp. (AM988942)	97.2	**10+ 0**	**10**					
Sediminibacterium salmoneum (EF407879)	96.9	**0 + 10**						**10**
Planctomycetes								
Blastopirellula sp. (EF419414)	96.3	**23 + 0**		**23**				
Singulisphaera acidiphila (AM902526)	92.9	**10 + 0**		**10**				
Schlesneria paludicola (AM162407)	89.9	**8 + 0**		**8****				
Phycisphaera mikrensis (AB474364)	83	**0 + 11**					**11****	
Candidate division OD1								
Uncult. bact. (AY922093)	90.9	**0 + 6**				**6****		
Eukaryotes								
***Bacillariophyta*, diatoma (cpDNA)**								
Skeletonema pseudocostatum (X82155)	90.7	**0 + 10**					**8****	**10****
***Viridiplantae*, green algae (cpDNA)**								
Chlamydomonas reinhardtii (FJ458262)	84	**84 + 54**	**20****		**84****	**6****		**54****
***Oomycetes* (mtDNA)**								
Phytophthora infestans (U17009)	73	**0 + 8**					**8****	
Saprolegnia ferax (AY534144)	73	**8 + 0**		**8****				

Notes: [1] considering the maximum value for each library;

* % of clones in a library;

** Unidentified (novel) phylotype; phylotypes shared by both water horizons are highlighted with gray shading.

The PCR amplicons (fragment sizes: 590, 890 and 1510 base pairs according to *E. coli* gene enumeration) were cloned using TOPO TA Cloning® Kit for Sequencing, Invitrogen, United States. The library coverage was estimated by clone ribotyping using three endonuclease enzymes, AluI, HpaII & HaeIII Fermentas, Lithuaina, and by clone sequencing. Representatives of each ribotype were sequenced by LGC Genomics GmbH (Berlin, Germany). Nucleotide sequence analyses were performed using the CLUSTALW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) software package and the BLAST algorithm of the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences were grouped into phylotypes using a threshold of ≥98% sequence similarity. The same value was used for phylotype identification amongst closest relatives in GenBank using BLAST. Sequences of the revealed phylotypes were deposited in the GenBank database under accession numbers JF901736–JF901755.

The microbial cell concentrations in the two water horizons were in the range $1.6–2.4 \times 10^4$ cell/ml which represents a rather low biomass.

In total, six clone libraries were constructed for samples R1 and R367: 0R and 3R (v3–v5 region of 16S rRNA gene), 17R and 37R (v4–v8 region), f1R and f3R (full-size gene), respectively. The clone numbers analyzed for each library were about 40 clones, except the 3R library where nearly 90 clones were studied. Some phylotypes were finally discarded as contaminants due to their presence in our Contaminant Library (sham DNA extraction, negative PCR, laboratory water etc.) [8].

All the data for three clone libraries were combined for the upper horizon sample R1 and revealed a total of 10 dominant phylotypes. Amongst them 8 phylotypes were assigned with 4 bacterial divisions (*Actinobacteria*, β-*proteobacteria*, *Bacteroidetes*, *Planctomycetes*), one-with chloroplast DNA of green algae, and the last one-with mitochondrial DNA of an oomycete (table). Three phylotypes showed <90% similarity to the nearest known taxon (planctomycete *Schlesneria paludicola*, green algae *Chlamydomonas reinhardtii*, and oomycete *Saprolegnia ferax*) and, thus, were referred further as unidentified, novel microbial species.

For the bottom layer sample R367, a total of 14 dominant phylotypes were recovered. Amongst them 11 were assigned to six bacterial divisions (*Actinobacteria*, α-*proteobacteria*, δ-*proteobacteria*, *Bacteroidetes*, *Planctomycetes*, Candidate division OD1), two phylotypes – with chloroplast DNA of green and diatom algaes, and one – with oomycete mitochondrial DNA. Similar to the above 6 phylotypes (distantly related to known taxa – the bacteria *Phycisphaera mikrensis* and *Desulfomicrobium hypogeium*, one environmental clone of Candidate division OD1, algae *Chlamydomonas reinhardtii* and *Skeletonema pseudocostatum* and oomycete *Phytophthora infestans*) were pooled into unidentified novel species (table).

All 6 clone libraries for both water layers came up with a total of 20 phylotypes with 7 phylotypes affiliated to bacterial divisions and 3 - with eukaryotic divisions. Amongst them only 4 phylotypes were detected in both water horizons: two phylotypes (clades acI-AVI and acI-AIV [11]) closely related to actinobacterium "*Candidatus* Planktophila limnetica" [10], one of *Mycobacterium vanbaalenii* (*Actinobacteria* as well) and one-representative of eukaryotes, very distantly (in fact unknown) related to green algae *Chlamydomonas reinhardtii*.

All these "shared" phylotypes except the clade acI-AIV actinobacterum were rather abundant in both horizons. Another 16 phylotypes appeared to be horizon -specific (6 - for the uppermost and 10 - for the bottommost horizons (table)) suggesting potentially significant microbial stratification of water column of the Lake Radok. The diversity in the bottom layer was higher than the uppermost layer, and this corresponds well with existing data [12].

It is noteworthy that the widely distributed acI gene pool of the recently described actinobacterium "*Candidatus* Planktophila limnetica" [10] accounted for in up to 44% and 48% of clones recovered in the uppermost and bottommost horizons, respectively (v3–v5 library, table) and these are known to be dominant elsewhere.

This could suggest evidence for a role in the structure and maintenance of the microbial community. The acI gene pool consists of three clades: acI-AVI, acI-AIV and third non-defined clade within the same acI-A subgroup.

The acI-AVI clade dominated the uppermost water horizon and (along with 'non-defined' clade) the bottommost horizon, whereas the acI-AIV clade was detected in both samples in insignificant numbers.

It is well known that the acI-AVI clade dominates bacterial freshwater communities and prefer slightly alkaline conditions unlike the other clades of the acI-A subgroup [11].

In conclusion, the microbial content and assemblages were determined for two depth-contrasting water horizons of Lake Radok. The results suggest significant microbial stratification of the Lake Radok water column with mainly distinct phylotypes. In both the uppemost and bottommost water horizons the actinobacterium *"Candidatus* Planktophila limnetica" as well as an unknown relative of the green algae *Chlamydomonas reinhardtii* was shown to be the key dominant components of the lake microbial community. In addition, at least two different regions (e.g., v3–v5 and v4–v8) of bacterial rRNA genes were needed to accurately describe the microbial diversity.

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