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Illuminating the microbial ecology of extreme saline meromictic lakes

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PhD Thesis Abstract

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Cluj-Napoca

2015

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Keywords: saline environments, meromictic lakes, sapropels, methanogenesis, metabolism, biogeochemical cycling, deep coverage SSU rDNA amplicon sequencing, prokaryotic diversity, ultrasmall uncultivated archaea

Chapter 1

The first chapter provides an overview of our current knowledge of halophilic microorganisms with a strong emphasis on the ecology of these extremophiles. As life on Earth subsists over the whole range of salt concentrations encountered in natural and anthropogenic habitats, halophiles (i.e. salt-loving organisms) thrive from saline soils and solar salterns to unexpected places like: salted fish, ancient mural paintings and even in the nostril salt glands of the sea bird *Calonectris diomedea*. They are found all over the small subunit rRNA-based tree of life and were mostly studied in costal solar saltern environments- artificial ponds constructed for the production of salt by evaporation of seawater. As our technology driven exploration of the tree of life is higher than ever before and our search for exo-Earths biosignatures is at its peak, the study of extremophiles arises as a prerequisite for the understanding of the processes that sustain life as we know it.

Chapter 2

The second chapter of the thesis focuses on the phylogenetic diversity and metabolic variability of halophilic Archaea. They include the heterotrophic haloarchaea (class *Halobacteria*) and some methanogenic Archaea, and inhabit both oxic and anoxic environments. In spite of their common hypersaline environment, halophilic archaea are surprisingly diverse in their nutritional demands, range of carbon sources degraded (including hydrocarbons and aromatic compounds) and metabolic pathways. The recent discovery of a new group of extremely halophilic *Euryarchaeota*, the yet uncultured *Nanohaloarchaea*, shows the archaeal diversity and metabolic variability in hypersaline environments is higher than hitherto estimated. Only recently we are starting to obtain a reliable picture of this diversity, which is much greater than the one predicted on the basis of culture methods. Research on the microbial diversity in hypersaline systems contributes greatly to our understanding of prokaryotic phylogeny, the adaptation of microorganisms to life under extreme conditions, and has substantial biotechnological implications.

Chapter 3

The third chapter examines the prokaryotic assemblages harbored by the heliothermal hypersaline meromictic Ursu and Fara Fund lakes (Transylvanian Basin, Romania). The prokaryotic assemblages from Ursu and Fara Fund hypersaline meromictic lakes (Transylvanian Basin, Romania) were compared in relation to their limnological factors and their role in elemental cycling was inferred by matching taxa to known taxon-specific biogeochemical functions. To assess the composition and structure of prokaryotic communities and the environmental factors that structure them, deep-coverage small subunit (SSU) ribosomal RNA (rDNA) amplicon sequencing, community domain-specific quantitative PCR and physicochemical analyses were performed on samples collected along depth profiles. The analyses showed that the lakes harbored multiple and diverse prokaryotic communities whose distribution mirrored the water stratification patterns. Ursu Lake was found to be dominated by Bacteria and to have a greater prokaryotic diversity than Fara Fund Lake that harbored an increased cell density and was populated mostly by Archaea within oxic strata. In spite of their contrasting diversity, the microbial populations indigenous to each lake pointed to similar physiological functions within carbon degradation and sulfate reduction. Furthermore, the taxonomy results coupled with methane detection and its stable C isotope composition indicated the presence of a yet-undescribed methanogenic group in the lakes' hypersaline monimolimnion. In addition, ultrasmall uncultivated archaeal lineages were detected in the chemocline of Fara Fund Lake, where the recently proposed *Nanohaloarchaeota* phylum was found to thrive.

Chapter 4

The fourth chapter concentrates on the sapropels retrieved from the analyzed hypersaline lakes. Sapropels are natural resources commonly used in curative and palliative care. However, despite the increasing exploitation and reserve diminution, their microbiological characterization lags behind. In this study, we described the prokaryotic communities from two disparate hypersaline sapropels (Transylvanian Basin, Romania) in relation to geochemical milieu and pore water chemistry, while inferring their role in carbon cycling by matching taxa to known taxon-specific biogeochemical functions. The polyphasic approach combined deep coverage SSU rDNA amplicon sequencing and bioinformatics with RT-qPCR and physicochemical investigations. We found that sapropels developed an analogous elemental milieu and harbored prokaryotes affiliated with fifty-nine phyla, among which the most abundant were *Proteobacteria*, *Bacteroidetes* and *Chloroflexi*. Containing thirty-two candidate divisions and possibly undocumented prokaryotic lineages, the hypersaline sapropels were found to accommodate one of the most diverse and novel ecosystems reported to date and might contribute to completing the phylogenetic foliage of the tree of life.

Chapter 5

The last chapter is primarily focused on exploring the factors responsible for the variation and distribution of microbial communities through the water column of Ursu and Fara Fund lakes, and extending the phylogenomic branching of the tree of life associated with these low-energy ecosystems. Hypersaline environments have proven useful as model systems in corroborating molecular (eco-)systems biology approaches and applications of ecological and evolutionary theory.

Furthermore, they serve as valuable models in establishing linkages between community structure and function, in uncovering the processes that determine the spatial structure of microbial assemblages and the mechanisms by which they respond to environmental changes. While microbial diversity studies based on 16S rRNA genes are highly informative and could provide valuable information regarding the community ecology and the environmental factors that structure its diversity, direct metagenomic sequencing of environmental samples can potentially provide additional functional data (missing from 16S rRNA gene surveys) and shed light on community-wide processes and ecosystem contributions of previously uncharacterized microorganisms. All in all, we showed that in Ursu and Fara Fund lakes the bacterial communities were structured by both neutral and species sorting processes, while the archaeal ones were mostly shaped by environmental filtering. Moreover, we assembled almost complete genomes of poorly investigated microbial lineages and provided the first genomic information about Parvarchaeota and OD1 clades that live in hypersaline systems. We also revealed that by utilizing metagenomic sequencing in combination with assembly strategies, novel microbes could be detected in scarcely investigated ecosystems and consider that similar studies carried out across various habitats will provide a more realistic view of the microbial world and will help us understand the extent of its diversity.