Protists not pickled by hypersalinity: Microbial eukaryotes in deep hypersaline anoxic basins (E. Mediterranean)
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The combination of nearly saturated salt concentration and corresponding high density, high hydrostatic pressure, absence of light, anoxia, and a sharp chemocline make the deep hypersaline anoxic basins in the Eastern Mediterranean Sea some of the most polyextreme habitats on Earth. However prior to our studies, it was unclear if protists were actually able to inhabit these habitats or if they were merely “pickled” carcasses. Our field studies have revealed unique microbial eukaryote communities living in the haloclines and brines of different basins with unique chemistries. These communities appear to be dominated by kinetoplastids, dinoflagellates, and ciliates. Fluorescent in situ hybridization (FISH) was used to visualize active cells corresponding to a novel ‘unidentified’ clade of kinetoplastids related to bodonids in the Discovery Basin halocline. FISH was also used to identify active dinoflagellate and ciliate populations in Urania and Discovery Basins. Scanning electron microscopy of Discovery and Urania halocline samples revealed abundant eukaryotic/prokaryotic partnerships that appear unique to each halocline, presumably due to the different hydrochemistries. Whole community metatranscriptomics provides insights into the activities of these eukaryotes in these polyextreme habitats. Our studies show that eukaryotic communities in these habitats include novel lineages, and that protists are capable of living under these seemingly adverse conditions.

Diversity of planktonic ciliates in deep hypersaline anoxic basins in the Eastern Mediterranean Sea
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Salt concentrations up to saturation, high pressure, anoxia, high methane and sulfide concentrations challenge basic processes of life in some Eastern Mediterranean deep-sea basins (DHABs – Deep Hypersaline Anoxic Basins). Yet, in the last few years molecular diversity surveys in these DHABs revealed initial proof for the existence of life from all three domains of life. In the domain Eukarya, ciliates, dinoflagellates and kinetoplastids belong to the dominant taxon groups of the protistan plankton. Using a next generation high throughput sequencing strategy we here compare the ciliate communities in different DHABs. Because each DHAB is characterized by a unique set of hydrochemical parameters and isolated from each other, these habitats are ideal model systems to study the influence of environmental selection on planktonic ciliate communities. We show that each basin is characterized by a unique ciliate community and identify environmental parameters explaining a large proportion of the observed spatial variability.
Environmental selection of protistan plankton communities in hypersaline anoxic deep-sea basins, Eastern Mediterranean Sea
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Deep hypersaline anoxic basins in the Eastern Mediterranean Sea are some of the most polyextreme habitats on Earth due to the combination of nearly saturated salt concentration, absence of light, anoxia, and high hydrostatic pressure. The geographical proximity and unique chemical characteristics of these basins make them ideal for testing hypotheses on influencing effects of environmental selection and distance on the shaping of protistan communities. T-RFLP analyses were performed on water samples from the brines and seawater/brine interfaces of five basins: Discovery, Urania, Thetis, Tyro and Medee. Analyses of similarities between the ten individual T-RF profiles, based on peak abundance and peak incidence, were used to assess the beta-diversity between brine waters with varying hydrochemistry and along geochemical gradients within oxic/anoxic interfaces. While a significant distance effect on protistan biogeography was not detected, geochemical gradients appear to act as dispersal barriers that likely lead to environmental selection in the DHAB protistan plankton communities. Hydrochemical barriers appear to be responsible for observed dissimilarities among protistan populations, with sodium, magnesium, sulfate and oxygen emerging as the dominant driving environmental factors.

Polyextremotolerant human opportunistic black yeasts inhabit dishwashers around the world
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Recent investigations have revealed that human households represent a suitable habitat not only for ubiquitous saprobic microorganisms, but also for extremotolerant microbes. Extreme human-made ecological niches inside the household are appliances such as washing machines and dishwashers. Predominant abiotic factors are high temperatures (up to 80°C), high pH (up to 12), high salinity (up to 20%NaCl) and high oxidative stress (different detergents). Our sampling of surfaces within 200 dishwashers in private homes in Europe, North and South America, Israel, South Africa, far East Asia and Australia revealed that almost two thirds of them were inhabited by extremotolerant fungi, which otherwise only thrive in special niches in nature. The consistent fungal community was primarily composed of different thermotolerant yeast species, reported to cause systemic diseases in immunocompromised patients and are known as causative agents of nosocomial infections. The most persistently and frequently isolated species were black yeasts Exophiala dermatitidis and Exophiala phaeomuriformis (Chaetothyriales). Both Exophiala species are known to be able to cause systemic disease in humans and frequently colonize the lungs of patients with cystic fibrosis. Their physiological characteristics, such as ability to grow at high temperatures, high moisture and alkaline pH, typically occurring in dishwashers, their special meristematic morphology and the ability of biofilm formation, enable their survival under the harshest conditions and transmission from dishwashers to the (water) environment. We concluded that the dishwashers provide an alternative habitat, selecting and enriching polyextremotolerant species. Almost daily exposure of people to aerosols and contaminated kitchenware from dishwashers might represent an important risk factor for immunocompromised people, small children and elderly, as well as for healthy individuals.
The most rapid climate warming is taking place at the highest northern latitudes and High Arctic lakes have been identified as sentinels of global change. The aim of this study was to compare microbial community structure across a range of High Arctic lakes in order to investigate their sensitivity to climate change. The planktonic communities from three lakes located along the northern coast of Ellesmere Island were studied in summer 2010 and 2011: meromictic lakes A (83°00’N, 75°30’W) and C1 (82° 51’ N, 78° 12’ W), and the epishelf lake of Milne Fjord (82° 45’ N, 82° 00’ W). Conductivity, temperature and oxygen concentration profiles were taken and phytoplankton communities were sampled throughout the water column for HPLC pigments and flow cytometry analyses. Pronounced differences in community composition were observed with depth down the three water columns. In Lake A and C1, picoeukaryotes (dinoflagellates, diatoms, cryptophytes, chrysophytes and chlorophytes) and their associated pigments (alloxanthin, fucoxanthin, violaxanthin, diadinoxanthin, lutein, chlorophylls c1, c2 and c3) occurred in the surface freshwater layer, while a deep maximum of bacteriochlorophyll-e associated with photosynthetic sulfur bacteria was found in lower waters that were anoxic and saline. Picocyanobacteria and zeaxanthin were observed throughout the water column. Milne Fjord showed a different pattern, with biomass dominated by picocyanobacteria in the surface freshwater layer, with chlorophyll b, violaxanthin and lutein-containing picoeukaryotes also present. Fucoxanthin-containing phytoplankton was observed deeper in the water column, but bacteriochlorophyll-e was absent. Such pigment and population diversity may provide an adaptive resilience to the shifting ice, mixing and underwater light conditions associated with climate change.

Antarctic lake ecosystems have an enormous potential for the discovery of novel biochemical pathways and physiological adaptations of microorganisms to extreme conditions. Lake Radok is the deepest (367.5m) perennally ice-covered freshwater, oligotrophic lake in Antarctica. The objective of this research was to study the microbial content and diversity of Lake Radok water column using bacterial 16S rRNA gene sequencing.

Here we report on microbial community diversity in 4 water column horizons collected in the deepest point of Lake Radok: the surface sample R1 (1.3m), the near bottom sample R367 (367m) and 2 intermittent horizons (R100 – 100m and R1 – 200m). The microbial cell concentrations in 4 water horizons were in the range 1.6-2.4 x 10⁴ cells/ml which represents a rather low biomass. The water samples were concentrated following genomic DNA extraction. To amplify bacterial 16S rRNA genes three pairs of degenerate universal PCR primers targeting the variable regions v3-v5, v4-v8 along with full-gene were used. For surface R1 and bottom R367 samples 3 regions were studied whereas for R100 and R200 samples only two of them were analyzed (v3-v5;v4-v8). The clones of total 10 libraries constructed were ribotyped with 3 restriction endonucleases before sequencing. Sequences were grouped into phylotypes using cutoff value ≥98% similarity.

All 10 clone libraries for all 4 water horizons came up with a total of 40 microbial phylotypes. Of them bacteria comprised 8 divisions while eukarya - 3 divisions including green algae, diatoms and oomycetes. Amongst the phylotypes recovered 33 were regarded as new ones showing less than 98% similarity with those in GenBank, and 14 phylotypes were considered as unidentified microbial taxa showing less than
90% similarity to known taxa. The predominant phylogenetic groups were Actinobacteria (40%) and green algae (22%) as viewed by all sequenced regions. Among others α-, β- and δ-Proteobacteria, Bacteroidetes, Verrucomicrobia, OD1 along with rather interesting phylum of Planctomycetes recovered by analyzing the only v4–v8 region of the gene were identified. Interestingly, that the gene pool consisting of three clades of acl-A subgroup of recently described actinobacterium “Candidatus” Planktophila limnetica, was predominated in 4 water horizons (varying from 19% till 40%).

Thus, Lake Radok water column despite of rather cool conditions features the fountain of microbial life comprising 40 microbial phylotypes. They were discovered by analyzing at least two different 16S rRNA gene regions which seems to be a prerequisite to assess the lake microbial diversity.

Subzero growth and adaptation; insights from Planococcus halocryophilus sp. nov. Or1 in Canadian high Arctic permafrost
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To date, the coldest reported temperatures for microbial growth and metabolism are -12°C and -32°C, respectively. In our investigations we have screened isolates from the Canadian high Arctic with several capable of subzero growth. Among these isolates we have recently described Planococcus halocryophilus sp. nov. Or1 representing 5.8% of the total bacterial community (by 16S rRNA pyrosequencing) in permafrost active layer from Eureka (Ellesmere Island, Canada). Here we present the sequenced genome, along with physiological and transcriptomic analyses that aim to uncover molecular characteristics required for subzero growth in hypersaline conditions. Growth characteristics and metabolic activity were determined in high salinity (up to 20 % NaCl) media over many weeks of incubation at subzero (-5, -10, -15, -20, -25 ºC) temperatures. P. halocryophilus Or1 was characterized as a eurypsychrophile growing up to 37 ºC and capable of subzero growth down to at least -15 ºC, tolerant of salinities up to 18 % NaCl, and metabolically active at -25ºC under frozen conditions. Genome sequencing was performed by both 454-pyrosequencing and Illumina and a 3.4 Mbp consensus sequence was assembled (Mira, Abyss) and annotated using automated pipelines (RAST) with manual validation. Genome-wide cold adaptation is apparent in the amino acid distribution favouring increased flexibility and stability of proteins by having less aliphatic and acidic residues. Synergistic adaptation to osmotic and cold stresses also appear on a genome scale with multiple copies of osmolyte uptake genes (for glycine and betaine), in higher abundance than in any other psychrotolerant bacteria.I genome. Transcriptomic analyses (Illumina) were used to compare the total genomic response to non-stress (25 ºC), high salt (18%), and low temperature (-15ºC) growth conditions. Transcriptomic results for optimal versus cold growth conditions indicated a small subset of genes (2% of coding sequences) with greater than 2-fold increases, several of which were involved in cell division, envelope processes, motility, and solute uptake including genes encoding peptidoglycan glycosyltransferase (FtsI), extracellular solute binding protein, membrane permeases, flagellar synthesis and related sigma factor (sigma-28). Unique cell envelope modifications are apparent in SEM and CLSM analyses at -15ºC that are distinct from those related to increased EPS production at growth temperatures down to -5ºC, and may be associated with dense lipid-rich material coating the cells at -15°C. Documenting the activity of extremophiles such as P. halocryophilus provides insight into molecular traits required for subzero growth and helps understand how viable cells function at the coldest growth temperatures described to date. The combination of flexibility and stability of proteins and the cell membrane compensate against the ordering/disordering effects of a cold and solute-rich environment while effective osmolyte uptake balances the osmotic pressure exerted over the cell envelope creating a solute-rich cytoplasm capable of remaining liquid at -15°C; all of which suggest that P. halocryophilus is suited for survival and growth in its Arctic permafrost habitat (~-16°C), indicating that these cryoenvironments harbor active microbial ecosystems.
Life in hypersaline environments could be limited by bioenergetic constraints. Microbial activity at the thermodynamic edge, such as the anaerobic oxidation of methane (AOM) coupled to sulphate reduction (SR) is thus unlikely to thrive in these environments. In this study, carbon and sulphur cycling were investigated in the hypersaline cold seep sediments of Mercator mud volcano (MV) and compared to the adjacent non-hypersaline Captain Arutyunov MV. AOM activity - albeit partially inhibited- was still present at a salinity of 263 g L\(^{-1}\) (circa 8 fold seawater concentration) with rates of 2.3 nmol cm\(^{-3}\) d\(^{-1}\), and even under saturated conditions with rates of 0.5 nmol cm\(^{-3}\) d\(^{-1}\). Methane and evaporite-derived sulphate co-migrated in the ascending fluid, which, in combination with a partial activity inhibition, resulted in an AOM activity that was spread over wide depth intervals. Up to 79\% of total cells in the AOM zone were identified as ANME-1 by fluorescence in situ hybridisation (FISH). No other ANME type could be detected either by FISH or in 16s rDNA gene libraries, indicating a possible salinity selection toward ANME-1. The depth distribution of methanogenesis rates did not match AOM rates nor ANME-1 cells counts, thus arguing for a strict methanotrophic role of these cells in Mercator MV sediments. Most ANME-1 cells formed monospecific chains without any attached partner. At all sites, AOM activity co-occurred with SR activity and sometimes significantly exceeded it. Possible causes of these unexpected results are discussed. This study demonstrates that in spite of a low energy yield, microorganisms carrying AOM can thrive in salinity up to halite saturation.