



Title	Molecular and morphological characterization of free-floating filamentous cyanobacterial mats from geothermal springs in the Philippines
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Citation	International Journal of Astrobiology, 2004, v. 4 n. S1, p. 67
Issued Date	2004
URL	http://hdl.handle.net/10722/42382
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isolated members of the genus *Psychrobacter*, a psychrotolerant bacterium not usually associated with mesophilic environments.

The bacteria isolated from the Siberian permafrost have been exposed to temperatures below 0°C for millions of years. It is expected that the traits that have been selected for in the permafrost habitat will not be as frequent in isolates from constantly warm environments. In order to test this hypothesis, we intend to compare various physiological traits between the bacteria isolated in the permafrost and the nearest relatives isolated from tropical environments. Several samples were taken from different ecosystems in Puerto Rico and bacteria were isolated using the media developed for the cultivation of *Psychrobacter* (Bowman et al. 1996). *Psychrobacter* specific 16S rRNA primers were used to identify positive isolates from fish and mangrove sediments. This presentation includes the preliminary description of *Psychrobacter* strains isolated from Puerto Rico, emphasizing the physiological comparison between the Puerto Rican and Siberian strains using traditional and molecular typing tools.

Reference

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Acknowledgements

We are grateful to Dr. Grizelle González and Mr. Andrés Lara for assistance in sample collection. This research was supported by grant NCC2-1274 from the NASA Astrobiology Institute.

Bacterially Mediated Ice Formation: Implications For Cloud Formation And Life In Frozen Environments

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Even though frozen habitats on Earth are known to contain liquid water due to salt and organic impurities that depress the freezing point in support of microbial life, surprisingly little is known about how cold-loving or psychrophilic bacteria interact and cope with the solid phase (ice) itself. Recent investigations of wintertime Arctic sea-ice samples have indicated that association with surfaces (including ice crystals) is an adaptive strategy for maintaining activity at subzero temperatures (to -20 °C, Junge et al., 2004). Questions remain, however, as to how bacteria can interact with ice directly.

In this pilot study, we investigated psychrophilic bacteria for their potential to induce ice formation, creating their own attachment sites, using a combination of microbiological and atmospheric science approaches in the laboratory. Ice nucleation activity (INA) of selected sea-ice bacterial isolates and their polymers, extracted from spent growth medium by ethanol precipitation and dialysis, were examined using the droplet freezing assay and a new freezing-tube technique. First tests revealed that many isolates, when present in marine growth medium, nucleated ice in the temperature range of -6 to -17°C. In many cases, droplets began to freeze at about -5°C, similar to the high ice-nucleation temperatures observed for terrestrial bacteria with INA. The extracted polymers also had the ability to nucleate ice at relatively warm temperatures but to a lesser degree. These preliminary results suggest that some ice-forming nuclei (IFN) in this population have INA at sufficiently high temperatures to be involved in both sea-ice and cloud formation. Future work will examine the implications for survival and growth of polar marine bacteria confronted with freezing conditions, as well as for bacterially mediated ice initiation in polar clouds on this planet and possibly elsewhere.

Reference

- K. Junge, H. Eicken, and J. W. Deming. 2004. Bacterial Activity at -2 to -20°C in Arctic Wintertime Sea Ice. *Appl. Environ. Microbiol.* 70: 550-557.

Acknowledgement

This research was supported by awards from the NSF OPP program to K. Junge and B. Swanson, and the NASA ABI to J. W. Deming and by the UW Astrobiology Program.

Biology And Water Chemistry Of Several Hyperalkaline Springs Emerging From Mantle-Like Rock

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Dilute (< 100 ppm TDS) hyperalkaline (pH > 11.5) springs associated with aquifers in the Cedars, a serpentinizing peridotite body in Northern California, appear to support a diverse microbiological community. The host rock, peridotite, is mantle-like in composition. When exposed to water near Earth's surface the minerals in peridotite tend to be converted to serpentine, i.e. the rock undergoes serpentinization. Peridotite was probably much more common on Earth's surface early on than today. Peridotite is also likely to be common on juvenile terrestrial planets in our solar system or around other stars of similar metallicity to Sol. Hence, water-rock-life interactions in this system may offer a window into such processes on early Earth or on other terrestrial bodies.

We have examined the water chemistry and microbiology of several different springs in this complex. We have successfully grown several cultures isolated from this environment at pH > 10 in the laboratory. We have also sequenced 16S rDNA genes from several springs and found that genetic diversity, though significant in all samples, is lowest in the most dilute and highest pH samples. This diversity is remarkable given that the water here is very dilute and contains almost no protons to drive PMF. According to ICP-MS analysis, these spring waters generally contain ≤ 2 mM Ca²⁺, Mg²⁺ and Na⁺ and much less of all other cations save Fe²⁺. Dissolved pCO₂ is <10⁻⁹ atm. Thus, as it emerges from the aquifers the water contains little in the way of either redox couples or carbon. When this water comes in contact with the atmosphere it literally sucks carbon dioxide out of the atmosphere, becoming supersaturated with respect to calcium carbonate.

Travertine aprons around many of the springs can grow tens of centimeters thicker in a single dry season though many aprons are partially or even completely destroyed during the rainy season. Given the enormous degree of supersaturation the water should reach, Cedars carbonates are most likely abiogenic in origin. However, initial examination suggests they could serve to preserve fossil microbes from this environment.

Acknowledgements

We wish to thank Roger Raiche and David McCrory for access to the Cedars and Robert Coleman for introducing us to the site.

Molecular and morphological characterization of free-floating filamentous cyanobacterial mats from geothermal springs in the Philippines

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A novel cyanobacterial mat type is characterized from near-neutral pH, low sulphide geothermal springs of 45-60 °C in the Philippines. Mats were free floating, several metres in diameter and several cm in thickness. The upper surface of mats was covered in a waxy scytonemin-like layer, solvent extracts of which absorbed light strongly at 384nm. Light microscopy revealed mats to possess highly ordered layers of air spaces at both the macroscopic and microscopic level, apparently as an adaptation to buoyancy. Morphospecies composition was exclusively filamentous, with Fischerella-like and Oscillatoria-like taxa closely associated throughout mats. Abundant heterocystous cells were observed in Fischerella filaments, suggesting nitrogen fixation occurs in these mats. Morphological structure did not vary among mats from pools of different temperature, but several 16S rDNA-defined genotypes were resolved by DGGE with some displaying greater thermophily than others. Sequencing of fourteen DGGE bands (Genbank accession numbers: AY236467-AY236480) yielded nine novel Fischerella sequences, whilst the five Oscillatoria sequences showed high similarity to other thermophilic Oscillatoria sequences. These data are relevant to astrobiology in that they expand our knowledge of oxygenic photosynthetic community diversity in geothermal environments, which serve as modern analogues for early life on Earth and other planets.

Acknowledgements

The authors are extremely grateful to the Philippine Institute of Volcanology and Seismology (PHIVOLCS) for advice and assistance with fieldwork. This work was supported by grants awarded by The University of Hong Kong CRCG Seed Funding for Basic Research and Small Projects programmes.