

Application of ribosomal intergenic spacer analysis (RISA) to analyze the diversity of halophilic Archaea in living stromatolites

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Hamelin Pool of Western Australia is one of the two places in the world, where living stromatolites can be found. These ancient laminated structures (age about 3.5 billion years) are mostly formed by cyanobacteria, but recent studies have uncovered a broad diversity of bacteria and Archaea in this habitat. The special environmental situation of Hamelin Pool (a shallow pool, contains twice the concentrations of Na⁺ and Cl compared to seawater) (1) allowed the Stromatolites to evolve over 3.5 billion years into the unique community of living organisms they are today. By understanding the structures and evaluating the diversity of these stromatolites we can get major insights into the formation of early life on Earth.

Until now, investigations of halophilic Archaea diversity of environmental samples were done by 16S rDNA sequencing. Recent publications emphasise the fact, that halophilic Archaea can have up to four different rRNA operons which can differ by up to 5 % in nucleotide sequence of the small subunit (2). This fact limits the use of 16S rDNA sequencing to investigate the diversity of halophilic archaea in environmental samples. RISA length polymorphism is widely used to identify bacteria and Archaea and also to determine diversity within environmental samples, but less is known about halophilic archaean profiles and profiles of high salt environments.

Cultures were isolated from Stromatolites and streaked several times to obtain pure cultures. Single pure colonies were picked and dissolved in 50 µl dH₂O. PCR was done with a fluorescence labeled primer (6 – fam labeled E 1492F) and the resulting products were separated over a sequencing capillary as previously described by Bornemann and Triplett (3). Previously, a RISA database from known halophilic archaea was generated and our isolates from Hamelin Pool were compared to this database.

Results indicate that RISA patterns are a useful method to discriminate halophilic archaea. Patterns of halophilic archaea seem to be consistent (Database samples were investigated twice) and novel isolates could be distinguished with this method. Further experiments target the possibility of using RISA specific PCR directly on environmental samples obtained from the stromatolites of Hamelin Pool.

- 1) Arp G., Reimer A. and Reitner J. Photosynthesis – Induced Biofilm Calcification and Calcium Concentrations in Phanerozoic Oceans. *Science* (2002); 1701-1704
- 2) Boucher Y., Douday C., Sharma A., Kamekura M. and Doolittle W. Intragenomic heterogeneity and intergenomic recombination among Haloarchael rRNA Genes. *Journal of Bacteriology* (2004); 3980 – 3990.

Bornemann J. and Triplett E. Molecular microbial diversity in soils from eastern amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl. Environ. Microbiol.* 63: 2647 – 2653