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METHOD PAPER

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Analysis of intergenic spacer region length polymorphisms to investigate the halophilic archaeal diversity of stromatolites and microbial mats

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Abstract Hamelin Pool in Western Australia is one of the two major sites in the world with active marine stromatolite formation. Surrounded by living smooth and pustular mats, these ancient laminated structures are associated with cyanobacterial communities. Recent studies have identified a wide diversity of bacteria and archaea in this habitat. By understanding and evaluating the microbial diversity of this environment we can obtain insights into the formation of early life on Earth, as stromatolites have been dated in the geological record as far back as 3.5 billion years. Automated ribosomal intergenic spacer analysis (ARISA) patterns were shown to be a useful method to genetically discriminate halophilic archaea within this environment. Patterns of known halophilic archaea are consistent, by replicate analysis, and the halophilic strains isolated from stromatolites have novel intergenic spacer profiles. ARISA–PCR, performed directly on extracted DNA from different sample sites, provided significant insights into the extent of previous unknown diversity of halophilic archaea within this environment. Cloning and sequence analysis of the spacer regions obtained from stromatolites confirmed the novel and broad diversity of halophilic archaea in this environment.

Keywords Shark Bay · Stromatolites · Intergenic spacer region · Diversity · Hypersaline environment · ARISA

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Introduction

The living stromatolites of Hamelin Pool, Shark Bay, in Western Australia are considered analogs of one of the oldest habitats of life on Earth (Monty 1977; Petrisor and Decho 2004; Reid et al. 2003). The surface of these stromatolites is covered with living mats which represent the actively growing microbial layer (Arp et al. 2001; Burns et al. 2004). Surrounding the stromatolites are living smooth and pustular mats, which may also have evolutionary significance because of their similarity to lithified stromatolites from the Proterozoic and Early Paleozoic eras (Palmisano et al. 1989). Due to the limited water exchange and the high evaporation rate in this area, the concentration of ions in the water is high, excluding most animals that would otherwise graze on the microbial mats. Despite these conditions in Hamelin Pool, including high levels of Na^+ and Cl^- (twice as high as normal seawater), recent research has revealed an extensive diversity of cyanobacteria and other bacteria (Burns et al. 2004; Papineau et al. 2005). Although the sodium chloride content of the water is only around 6% (w/v), the work by Burns et al. (2004) also revealed the presence of halophilic archaea of the family Halobacteriaceae. This diversity of as yet uncultivated halophilic archaea was surprising since these organisms grow optimally in media containing 15–35% (w/v) NaCl (Kushner and Kamekura 1988) and require at least a minimum of 9% (w/v) NaCl (Oren 2001) for active growth.

Halophilic archaea typically thrive in high salt environments and have been isolated from the Dead Sea (Oren et al. 1995), salt evaporation lagoons and ancient halite (McGenity et al. 2000; Mormile et al. 2003; Stan-Lotter et al. 2002). These environments are characterized by high ionic concentrations, and even halophiles isolated from seawater (Rodríguez-Valera et al. 1979)

require 2 M NaCl for growth. Recent publications have raised the possibility of cultivating halophilic archaea from low salt environments. For example, Purdy et al. (2004) cultivated three novel types that were isolated from 3.2% (w/v) NaCl environments and are also capable of slow growth at sea water salinity of 2.5% (w/v) NaCl.

Screening early Earth analogs, such as the Shark Bay stromatolites, for halophilic archaea can provide novel insights into possible early life formation since halophiles are known for their longevity and their ability to survive for several million years in ancient halite (Grant et al. 1998; Radax et al. 2001; Stan-Lotter et al. 2002). Furthermore, species of the genus *Halococcus* show an a previously unknown breadth of archaeal diversity indicated by intergenic spacer region polymorphisms.

Materials and methods

The following cultivated strains were obtained from Professor Masahiro Kamekura for ARISA analysis: *Haloterrigena turkmenica*, *Natrialba aegyptia*, *Halogeometricum borinquense*, *Halobaculum gomorrense*, *Halococcus salifodinae*, *Halococcus morrhuae*, *Halococcus saccharolyticus*, *Haloferax volcanii*, *Natrinema pallidum* and *Halorubrum trapanicum*. *Halobacterium salinarum* NRC-1 was obtained from Prof. Stan-Lotter. All strains