



The living rocks of Shark Bay

Stromatolites have been present on Earth, at various levels of distribution and diversity, for more than 3 billion years. Stromatolites are sedimentary structures produced by the sediment-trapping, binding and/or precipitation activity of resident microbial communities, in particular cyanobacteria.

Today, the only known examples of stromatolites forming in hypersaline coastal environments are in Hamelin Pool at Shark Bay, Western Australia (Figure 1). Due to the restricted flow of seawater into Hamelin Pool and high net evaporation rates, surface waters have salinity twice that of normal seawater¹. By analogy, many ancient stromatolites have been considered to form in hypersaline or intertidal conditions². Many important steps in evolution may have occurred within stromatolites owing to the close proximity of diverse microorganisms and microniches³. Thus modern stromatolites are a significant resource for studying the origin, evolution, and distribution of life, particularly the physiological processes that may leave preserved biosignatures in fossils on Earth and, potentially, distant biospheres.

Despite their evolutionary significance, until now little was known about the associated microbial communities of the Shark Bay stromatolites.

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Surfaces of stromatolites are covered with living mats, and our work focused on the microbial community of this actively growing layer as, at the microscale most relevant to bacteria, a vital factor that affects stromatolite formation is the presence of other organisms. Apart from physical factors such as wave action, stromatolite morphology will depend on the community present and will therefore be determined by it. The environment selects for biological diversity, which in turn is reflected in these biogenic structures. These stromatolites are still growing at a rate of about 0.4 mm year, and growth rates only slightly surpass erosion.

Our study is the first to combine culture-dependent and culture-independent methods in a study of this environment. After obtaining samples of the intertidal stromatolite, they were crushed and used directly as the inoculums in a range of microbiological media using conditions

suitable for the growth of cyanobacteria, heterotrophic bacteria and archaea. The salt concentrations for the different media were modified to levels to mimic the elevated environmental concentrations found in Shark Bay¹.

In addition to these cultivation experiments, we also constructed 16S rDNA cyanobacterial, eubacterial and archaeal clone libraries amplified directly from environmental genomic DNA extracted from the columnar stromatolite. Although culture-independent molecular analyses alone do not allow us to absolutely determine whether sequences represent active stromatolite organisms, we can take advantage of phylogenetic affinity with well-studied species to make predictions about the metabolic contributions of organisms we have identified.

The community was dominated by organisms of the cyanobacterial genera *Synechococcus*, *Xenococcus*, *Microcoleus*, *Leptolyngbya*, *Plectonema*, *Symploca*, *Cyanothece*, *Pleurocapsa* and *Nostoc*, including two novel groups that did not cluster with any known phyla. The majority of cyanobacterial isolates, were filamentous (Figure 2), a characteristic known to aid sediment trapping in stromatolites⁴. Many of the organisms identified here have also been associated with microbial mat formation in a range of environments^{4,6}, and their individual and combined physiologies would be expected to play critical roles in stromatolite construction at Shark Bay.

A number of the clones were identified as the cyanobacterium *Prochloron*. *Prochloron* is symbiotic with didemnid ascidians and to date there is no report of its existence as a free-living organism⁷. As no ascidians have been reported in Hamelin Pool, the observation of apparently free-living *Prochloron* associated with stromatolites here is of considerable interest.



Figure 1. Intertidal stromatolites, Hamelin Pool, WA.

The other eubacterial isolates and clones clustered into seven phylogenetic groups: OP9, OP10, Marine A group, Low G+C Gram-positive, *Planctomyces*, *Acidobacteria* and *Proteobacteria*. Though many of these eubacteria identified here may be non-structural community members, the presence of the latter group, in particular, is consistent with other studies revealing the diversity of *Proteobacteria* in microbial mats and their roles in biosedimentation.

We also demonstrated the presence of sequences corresponding to members of halophilic archaea of the divisions *Euryarchaeota* and *Crenarchaeota*, and methanogenic archaea of the order *Methanosarcinales*. This is the first report of such archaeal diversity from this environment. The majority of the archaeal clones identified were closely related to the Halobacteria, an archaeal class known to be abundant in hypersaline settings⁸. The presence of halophilic archaea in some abundance in our clone library suggests that forms of phototrophic metabolism other than cyanobacterial oxygenic photosynthesis

may contribute to the morphogenesis of the Shark Bay stromatolites.

At first one might consider that the specialised niche of the Shark Bay stromatolites supports a restricted range of microorganisms. However, our findings have demonstrated an array of metabolically diverse prokaryotes. The extremely slow water exchange in Shark Bay would reduce microorganism migration fluxes by advective losses. Hypersalinity appears to be a factor of prime importance in the development of these stromatolites, and this is reflected in the types of microorganisms we have identified. Many of the cloned populations are unique phylotypes, with no close relatives in the database, and the organisms represented by these clones may also possess novel physiologies vital to the persistent morphogenesis of Shark Bay stromatolites.

Oxygen, metabolites, and photosynthetically active light gradients that form throughout the stromatolite will determine the spatial arrangement of specific groups of organisms⁹. Their close

association in this setting may also facilitate horizontal gene transfer of evolutionally significant traits, including salt tolerance or antibiotic resistance. The latter properties could also be potentially exploited for biotechnological use.

In terms of management of this unique world resource, this work can be appreciated both from an applied and a conservation point of view. Though stromatolites have existed on this planet for three quarters of its existence, they are very vulnerable to human impact. To ensure their survival, the organisms that construct them must be identified and understood, which is the underlying foundation of our research. This research would be of significant interest in various fields such as biochemistry, molecular biology/genetics, geology and ecology, all encompassing the exciting emerging field of astrobiology.

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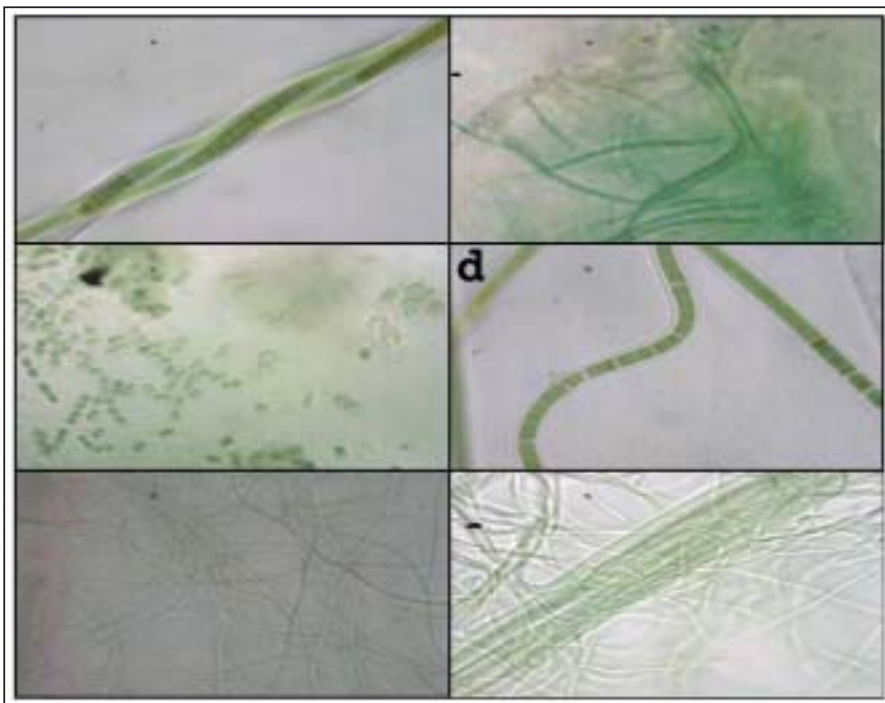


Figure 2. Diversity of cyanobacterial morphotypes identified in the stromatolites (Courtesy: F. Goh).
 (a) *Leptolyngbya* sp. (b) *Plectonema* sp.
 (c) *Xenococcus* sp. (d) *Symphloca* sp.
 (e) *Nostoc* sp. (f) *Cyanothece* sp.