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Prokaryotic Community Analysis of a Hyperalkaline Spring in the Philippines Using 16S rRNA Gene Clone Library Construction

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The prokaryotic diversity associated with serpentinization-driven Manleluag Hyperalkaline (pH 11) Spring in Pangasinan, Philippines was investigated. DNA extracted directly from the sediment samples was used to construct clone libraries based on bacterial and archaeal 16S rRNA gene sequences. Phylogenetic analysis of 16S rRNA gene sequences from the clone library revealed that the clones were grouped into *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Cyanobacteria*, *Bacteroidetes*, and *Firmicutes*. Analysis of the archaeal 16S rRNA clones revealed the presence of sequences associated with members of *Euryarchaeota* and *Thaumarchaeota*. Most of the sequences from *Euryarchaeota* were related to *Methanobacteria* and *Methanomicrobia*. Some clones show little affiliation with known taxa and may represent novel sequences of organisms adapted to the hyperalkaline conditions. The populations found suggest the type of metabolisms that drive this specific environment, which include ammonia oxidation, and hydrogen-based and methanogenic metabolisms. This study represents the first analysis of prokaryotic diversity from community DNA of a hyperalkaline environment in the Philippines.

Key Words: 16S rRNA gene sequence, alkaliphiles, hyperalkaline spring, phylogenetic analysis, serpentinization

INTRODUCTION

Natural nonsaline alkaline environments are not common while saline alkaline soda lakes and soda deserts have been systematically studied. Nonsaline alkaline environments are much rarer and their microbial populations have not been well-documented. The genesis of nonsaline alkaline environments is related to a geochemical process known as serpentinization (Tiago et al. 2004). This process is exothermic and large quantities of hydrogen gas, methane and low-molecular weight organic compounds can emanate from these serpentinizing regions (Brazelton *et* al. 2011). Serpentinization is therefore a potential source of reducing power and organic carbon for organisms inhabiting the ultramafic subsurface (Brazelton et al. 2012).

A unique natural alkaline environment in the Philippines is the Manleluag Hyperalkaline Spring, Pangasinan which is characterized by hyperalkaline (pH 10-11.5) and highly

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