

## Vertical distribution and phylogenetic characterization of Archaea in an anoxic shallow terrestrial subsurface Holocene sediment from the catchment of Lake Kitaura

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### Introduction

Studies on the diversity of Archaea in non-extreme environments such as soils and sediments are limited. Culture independent molecular analysis of archaeal DNA retrieved from an anoxic **Holocene sediment core** from Lake Kitaura catchment was performed by using 16S rRNA gene clone library analysis. Archaeal communities were analyzed at three depths; 2.1m, 3.6m and 6.1m varying in geochemical, geological and hydrological characteristics. The identification and phylogenetic placement of archaea from anoxic sediments is expected to contribute to our understanding of the ecological significance of Archaea in non-extreme environments.

### Materials and methods

Community DNA of was directly extracted from sediment core by using MOBIO Ultra clean Soil kit (Mega Prep) with an additional wash protocol. 16S rDNA were PCR amplified using primers specific for the Domain *Archaea* (primers ARC341F and ARC915R), the PCR products were cloned and sequenced. Sequences of approximately 450bp length were aligned using automatic nucleic acid aligner CLUSTAL W software package (EMBL-EBI). Phylogenetic trees were created using software package MEGA 2.1 (3).

### Results and Discussion

Phylogenetic analysis of cloned small subunit ribosomal RNA gene sequences revealed very low archaeal diversity in all three depths. No significant differences were found between the three communities between different depths. None of the archaeal sequences had above 98% similarity to a cultured archaeal representative. Most Archaeal sequences branched deeply within the **Miscellaneous Crenarchaeota Group (MCG)**(2), which has been detected widely in terrestrial and marine habitats. Majority of archaeal clones were closely related to representatives retrieved from the **deep subsurface Paleosol** of DOE Hanford site (1), followed by representatives from **subsurface geothermal water** stream in a Japanese gold mine (Hirayama et al., unpublished) and volcanic ash layers of the coastal **subseafloor sediments** from the sea of Okhotsk (2). It may not be possible to infer physiological and metabolic features of these archaea in the absence of cultivated representatives. But it is evident that the different environmental conditions that exist in different depths have not influenced the archaeal community structure significantly.

1. Chandler DP, FJ Brockman, TJ Baily, JK Fredrickson. 1998. *Microbial Ecol* 36, 37-50
2. Inagaki F, M Suzuki, K Takai, H Oida, T Sakamoto, K Aoki, KH Nealson, K Horikoshi. 2003. *Appl Environ Microbiol* 69, 7224-7235
3. Kumar S, K Tamura, IB Jakobsen, M Nei. 2001. *Bioinformatics* 17, 1244-1245  
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