Archaeal Diversity in Tidal Flat Sediment as Revealed by 16S rDNA Analysis

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During the past ten years, *Archaea* have been recognized as a widespread and significant component of marine picoplankton assemblages. More recently, the presence of novel archaeal phylogenetic lineages has been discovered in coastal marine environments, freshwater lakes, polar seas, and deep-sea hydrothermal vents. Therefore, we conducted an investigation into the archaeal community existing in tidal flat sediment collected from Ganghwa Island, Korea. Phylogenetic analysis of archaeal 16S rDNA amplified directly from tidal flat sediment DNA revealed the presence of two major lineages, belonging to the *Crenarchaeota* (53.9%) and *Euryarchaeota* (46.1%) phyla. A total of 102 clones were then sequenced and analyzed by comprehensive phylogenetic analysis. The sequences determined in our samples were found to be closely related to the sequences of clones which had been previously obtained from a variety of marine environments. Archaeal clones exhibited higher similarities (83.25 - 100%) to sequences from other environments in the public database than did those (75.22 - 98.46%) of previously reported bacterial clones obtained from tidal flat sediment. The results of our study suggest that the archaeal community in tidal flat sediment is remarkably diverse.

Key words: 16S rDNA, archaeal community, phylogeny, tidal flat sediment

16S rDNA sequence-based molecular phylogenetic analysis has been used extensively to understand community biodiversity, structure and functionality of a variety of ecosystems. The culture-independent method, such as sequencing of 16S rDNA from clone libraries of DNAs from environmental samples, provides an alternative means for the elucidation of bacterial community structures. This strategy has also been employed in order to overcome the limitations of traditional cultivation methods, which are insufficient for the culturing of most *Bacteria* or *Archaea* (Torsvik *et al.*, 1996; Hugenholtz *et al.*, 1998; Lee *et al.*, 1999; Cho *et al.*, 2003). Sequence data can also be used to compare community structures in different environments (Hur and Chun, 2004).

Among three major evolutionary domains of life on Earth, members of the *Archaea* domain are the least understood in terms of their diversity, physiology, genetics, and ecology. *Archaea* domain is divided into four phyla, *Euryarchaeota*, *Crenarchaeota*, *Korarchaeota*, the presence of which has been determined only by environmental DNA sequences (Barns *et al.*, 1996; Bano *et al.*, 2004), and the recently reported *Nanoarchaeota* (Huber *et*

al., 2002). Molecular phylogenetic studies have revealed that environmental archaeal populations are diverse, complex and widespread, and that they frequently consist of uncultivated and unidentified members. As it is currently impossible to construct culture-based phenotypic characterizations of many environmental *Archaea*, the physiological significance of *Archaea* in nature has remained unknown for a long time. When the phylogenetic features intrinsic to archaeal communities are related to the environment, they may provide important insights into the physiological functions and ecological roles of communities (Takai *et al.*, 2001). Several recent molecular studies (Bintrim *et al.*, 1997; Jurgens *et al.*, 1997; Buckley *et al.*, 1998) have demonstrated the ubiquity of *Archaea* in soil, particularly those organisms belonging to the non-thermophilic *Crenarchaeota* lineage which forms a deeply branching group with no close affiliation with any cultivated member of *Archaea*. These organisms may constitute approximately 1% of the total soil population (Buckley *et al.*, 1998; Sandaa *et al.*, 1999).

The West and Southwest coasts of the Korean peninsula consist primarily of tidal flats, which are also known as getbol. Getbol are unique among other marine sediments as they are flooded and periodically exposed by seawater. A high degree of water temperature and salinity changes has been frequently observed in getbol. Getbol have been

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determined to be a hugely dynamic areas in terms of sediment erosion and deposition, and they can be distinguished from salt marsh and wetland by the degree to which these characteristics are observed (Carling, 1982). We previously reported the diversity of *Bacteria* in tidal flat sediment, Ganghwa Island (Kim *et al.*, 2004). This observation led to our increased interest in the community structure of *Archaea* in tidal flat. The objective of this study is to investigate the community structure and phylogenetic diversity of *Archaea* in tidal flat sediment of Ganghwa Island. This information may help to establish a framework for future study regarding the ecology of microorganisms in tidal flat sediments.

Materials and Methods

Sample collection and chemical analysis
Getbol sediment soils were obtained at a site in Dongmak (37°35.319'N, 126°27.245'E), on Ganghwa Island. Section from depths of 5 cm, was subsampled and stored in polypropylene bags. The samples were immediately stored on dry ice for transport to the laboratory. The samples were then stored at -80°C until analysis. The chemical properties of sampling site have been previously described (Kim *et al.*, 2004).

DNA extraction and PCR amplification
DNA extraction was followed by the carrying out of CsCl density equilibrium gradient methods, according to the direct lysis protocol for bacterial community DNA recovery (CsCl) (Hurst, 1997). The extracted DNA was then visualized on ethidium bromide-stained 1% agarose gel. We then performed PCR amplification of archaeal 16S rDNA from getbol DNA extracts, in the GeneAmp 9600 PCR system (PE Applied Biosystems, USA) at a total volume of 50 μl. Archaeal 16S rDNAs were enzymatically amplified with the archaeal-specific primers, 21F (5'-TTCCGGTTGATCCY-GCCGGA-3') and 958R (5'-YCCGGCGTTGAMTCCAA-TT-3') as previously described (DeLong, 1992). The PCR amplification conditions were as follows: 50 μl of total volume, 20 pmol of each primer, 2 U of Taq polymerase (Takara, Japan), 2/25 volume of dNTP, and a 1/10 volume of 10x Taq buffer were provided with the enzyme. After a denaturation step of 5 min at 94°C, amplification reactions were performed with 30 cycles of denaturation (1 min, 94°C), primer annealing (1 min, 55°C), and primer extension (1 min, 72°C), with a final 7 min extension step at 72°C. We observed no amplified products in the negative control reaction, and obtained an amplified product of the expected size (0.9 kb) when genomic DNA isolated from the sediments was used as a template.

Cloning and colony PCR amplification
PCR products were purified with a PCR Clean-up Kit (Mo Bio, USA). The purified 16S rDNA amplicons were then ligated into pGEM-T easy vector (Promega, USA), and transformed into *E. coli* DH10B cells. Colony PCR was then carried out as mentioned previously. The primers used were prGTf (5'-TACGACTCACTATAGGGC-GA-3') and prGTr (5'-CTCAAGCTATGCATCCAAC-GC-3'), which target the flanking regions of the multicloning site of pGEM-T easy vector (Chun *et al.*, 1999).

Amplified rDNA restriction analysis (ARDRA)
In order to determine the ARDRA patterns, 1 μl of colony PCR product was digested using two four-base-specific restriction enzymes (*Hha* I, *Hae* III) (NEB, USA) for 2 h at 37°C. The resultant fragments were analyzed by electrophoresis in 2% agarose gels (APB, USA). A 100 bp ladder (APB, USA) was used for a DNA marker.

Sequencing and phylogenic analysis of 16S rDNAs
The PCR products were purified with shrimp alkaline phosphatase and *Exo* I treatment, subsequent to the running of the ABI PRISM SnaPshot Multiplex Kit Protocol (Kim *et al.*, 2004). The sequences were then determined on an automated DNA sequencer (ABI Gene Scan 3100, USA), using the 21F primer. The clones were checked for chimeric artifacts with the CHECK_CHIMERA program, provided by the Ribosomal Database Project (RDP) (Maidak *et al.*, 1997). The results of initial comparisons of the sequences with the GenBank nonredundant database, using the BLAST program provided by the National Center for Biotechnology Information (NCBI) (available at http://www.ncbi.nlm.nih.gov/BLAST/), constituted a guide for the determination of which 16S rRNA sequences should be used in the sequence alignments. The calculation of sequence similarity and the phylogenetic tree inference were carried out using the jPHYDIT program (available at http://chunlab.snu.ac.kr/jphydit/). Similarity matrices were constructed via pairwise analysis, and evolutionary distance matrices were generated according to the method described by Jukes and Cantor (Jukes and Cantor, 1969). Phylogenetic trees were then constructed via neighbor-joining (Saitou and Nei, 1987). A bootstrap analysis (Felsenstein, 1985) was performed, in 1000 trial replications, in order to provide confidence estimates for the topology of phylogenetic tree. 12 clones of this study were redundant, and so were not submitted to the GenBank database. The sequences determined in this study were submitted to the GenBank database, and are designated by the accession numbers AY396615-AY396704.

Results

Sample analysis and amplified rDNA restriction analysis (ARDRA)
Genomic DNA was directly extracted from the getbol sediment via the CsCl density equilibrium gradient method, which has been used extensively for the extrac-

tion of DNA from humic acid-rich samples for PCR amplification (Holben *et al.*, 1988). The archaea-specific primers were used to successfully amplify the 16S rRNA genes. Archaeal clone library was constructed for the tidal flat sediment, and a total of 102 insert-containing clones were identified via direct PCR screening.

The ARDRA patterns were employed to initially measure strain diversity in the getbol. However, the clones were so diverse that most of them (80 clones of 102 clones) could not be clustered by the ARDRA patterns (data not shown). Therefore, all of the clones were examined via sequencing.
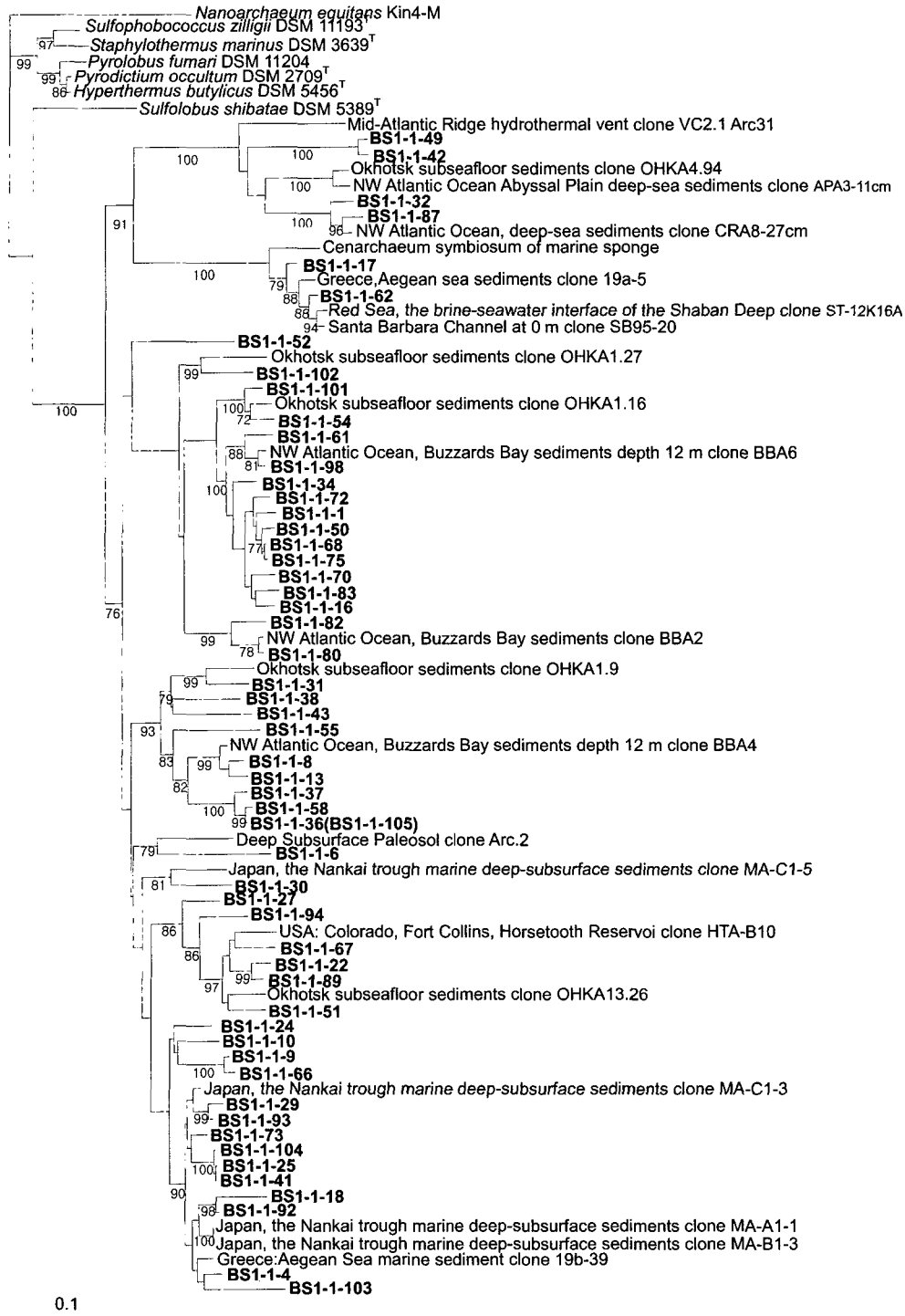
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                    Nanoarchaeum equitans Kin4-M
          Sulfophobococcus zilligii DSM 11193ᵀ
       97    Staphylothermus marinus DSM 3639ᵀ
    99    Pyrolobus fumari DSM 11204
        99  Pyrodictium occultum DSM 2709ᵀ
        88 Hyperthermus butylicus DSM 5456ᵀ
                    Sulfolobus shibatae DSM 5389ᵀ
                              Mid-Atlantic Ridge hydrothermal vent clone VC2.1 Arc31
                                BS1-1-49
              100         100     BS1-1-42
                              Okhotsk subseafloor sediments clone OHKA4.94
                           100    NW Atlantic Ocean Abyssal Plain deep-sea sediments clone APA3-11cm
                                BS1-1-32
                           100    BS1-1-87
          91                  96   NW Atlantic Ocean, deep-sea sediments clone CRA8-27cm
                              Cenarchaeum symbiosum of marine sponge
              100           BS1-1-17
                        79   Greece,Aegean sea sediments clone 19a-5
                        88  BS1-1-62
                         88  Red Sea, the brine-seawater interface of the Shaban Deep clone ST-12K16A
                           94  Santa Barbara Channel at 0 m clone SB95-20
                    BS1-1-52
                       Okhotsk subseafloor sediments clone OHKA1.27
                99      BS1-1-102
                        BS1-1-101
                    100   Okhotsk subseafloor sediments clone OHKA1.16
                     72   BS1-1-54
                        BS1-1-61
                     88   NW Atlantic Ocean, Buzzards Bay sediments depth 12 m clone BBA6
100                    81  BS1-1-98
                   100    BS1-1-34
                          BS1-1-72
                          BS1-1-1
                          BS1-1-50
                      77  BS1-1-68
                          BS1-1-75
                          BS1-1-70
                          BS1-1-83
                          BS1-1-16
                        BS1-1-82
76                99     NW Atlantic Ocean, Buzzards Bay sediments clone BBA2
                      78  BS1-1-80
                       Okhotsk subseafloor sediments clone OHKA1.9
                  99      BS1-1-31
                  79      BS1-1-38
                          BS1-1-43
              93        BS1-1-55
                       NW Atlantic Ocean, Buzzards Bay sediments depth 12 m clone BBA4
                83  99  BS1-1-8
                          BS1-1-13
                82        BS1-1-37
                      100  BS1-1-58
                      99 BS1-1-36(BS1-1-105)
                       Deep Subsurface Paleosol clone Arc.2
              79            BS1-1-6
                       Japan, the Nankai trough marine deep-subsurface sediments clone MA-C1-5
              81        BS1-1-30
                        BS1-1-27
                        BS1-1-94
                86        USA: Colorado, Fort Collins, Horsetooth Reservoi clone HTA-B10
                          BS1-1-67
                86        BS1-1-22
                    99   BS1-1-89
                97     Okhotsk subseafloor sediments clone OHKA13.26
                        BS1-1-51
                      BS1-1-24
                      BS1-1-10
                        BS1-1-9
                100     BS1-1-66
                    Japan, the Nankai trough marine deep-subsurface sediments clone MA-C1-3
                      BS1-1-29
                99  BS1-1-93
                      BS1-1-73
                      BS1-1-104
                    100 BS1-1-25
                      BS1-1-41
                90      BS1-1-18
                  98  BS1-1-92
                    Japan, the Nankai trough marine deep-subsurface sediments clone MA-A1-1
                    100 Japan, the Nankai trough marine deep-subsurface sediments clone MA-B1-3
                    Greece:Aegean Sea marine sediment clone 19b-39
                      BS1-1-4
                        BS1-1-103
    0.1
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Fig. 1. Phylogenetic tree based on the 16S rDNA sequences of the *Crenarchaeota* clones obtained from tidal flat sediment. The tree was constructed via neighbor-joining. *Nanoarchaeum equitans* Kin4-M was used as the outgroup. The percentage numbers at the nodes indicate the bootstrap support levels, based on neighbor-joining analyses of 1,000 resampled data sets. Bar, 10% nucleotide changes per 16S rDNA position.

Archaeal 16S rDNA clone library analysis

Archaeal 16S rDNA clones were characterized by partial sequencing (the average length of the sequence was 640 bases) and phylogenetic analysis. There were 105 total clones. Using the RDP' CHECK_CHIMERA program, 3 clones were determined to be probable chimeric amplicons, and these were omitted from the final analysis. All

clones were determined to belong to the phyla *Crenarchaeota* and *Euryarchaeota*, whereas none of clones were found to be members of either *Korarchaeota* or *Nanoarchaeota*.

In order to acquire an accurate description of the phylogenetic relationships of tidal flat sediment clones, we included representative sequences of both cultivated and
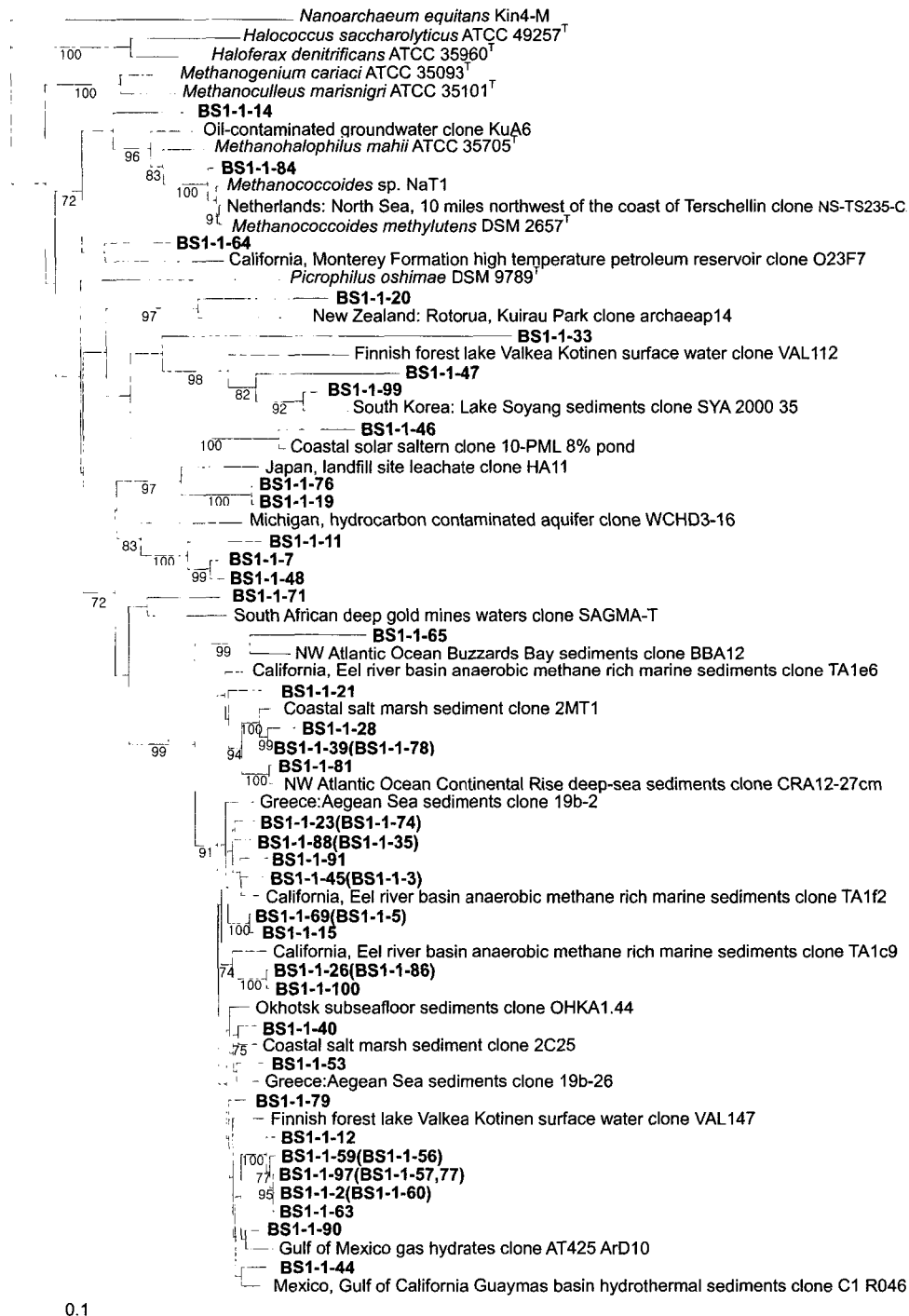
Fig. 2. Phylogenetic trees of the *Euryarchaeota* clones from tidal flat sediment. *Nanoarchaeum equitans* Kin4-M was used as the outgroup. Bar, 10% nucleotide changes per 16S rDNA position.

uncultivated archaeal clones in our analysis. In general, archaeal clones exhibited higher similarities (83.25 - 100%) to sequences from other environments in the public database than those (75.22 - 98.46%) of previously reported bacterial clones from tidal flat sediment (Kim *et al.*, 2004).

Phylogenetic analysis, using the neighbor-joining method, consistently placed 55 tidal flat sediment clones within the phylum *Crenarchaeota* (Fig. 1). On the basis of phylogenetic inference, these crenarchaeal sequences from getbol were determined to form a coherent clade, and were most closely associated with 16S rDNA clones obtained previously from a variety of marine environments (Brehmer, unpublished; Vetriani *et al.*, 1998, 1999; Massana *et al.*, 2000; Reysenbach *et al.*, 2000; Eder *et al.*, 2002; Reed *et al.*, 2002; Inagaki *et al.*, 2003) and freshwater reservoirs (Stein *et al.*, 2002). None of the clones were closely related with the cultivated archaeal 16S rDNA sequences in the public database. Six of the clones (BS1-1-17, -32, -42, -49, -62, -87) were associated with clones and isolates from deep-sea sediments (VC2.1 Arc31, APA3-11, OHKA4.94 and 19a-5) which had previously been isolated from a variety of ocean sediments. Clone BS1-1-87 exhibited a 97.3% similarity with the CRA8-27 clone, isolated from Northwest Atlantic Ocean deep-sea sediment (GenBank # AF119118). Clone CRA8-27 shares common ancestry with other benthic marine groups (Vetriani *et al.*, 1999). The remainders of the clones (49 clones) were clustered with clones from anoxic marine sediments, miscellaneous crenarchaeotic group sequences, and clones from methane hydrate-bearing subseafloor sediments, including those from the Nankai Trough in Japan. Clones BBA2, 4, and 6, all of which were obtained from anoxic, sulfide-rich marine sediments in Buzzards Bay, in the Northwest Atlantic Ocean (Vetriani *et al.*, 1998). The OHKA 1.27, 1.16, 1.9, and 13.26 clones from Okhotsk subseafloor sediments in Japan belonged to the Miscellaneous Crenarchaeotic Group (MCG) (Inagaki *et al.*, 2003).

Clone BS1-1-52 was only distantly related to the other sequences, and represented a deep branch. The highest similarity value of this clone was 89.37%, with the BBA6 clone from the NW Atlantic Ocean Buzzards Bay sediments (GenBank # AF004345). Nine clones (BS1-1-1, -16, -34, -50, -68, -70, -72, -75 and 83) were determined to form a monophyletic clade, and were also determined to be distantly related to other archaeal sequences, both cultured and uncultured. *Archaea* that belong to this crenarchaeotal clade may be widely spread throughout anoxic sediments, marine benthic sediments, and deep-sea hydrothermal vents. The highest similarity value in the *Crenarchaeota* phylum was found between clone BS1-1-80, and the Buzzards Bay sediments clone BBA2 (GenBank # AF004343, 100% similarity) (Vetriani *et al.*, 1998).

47 clones were determined to belong to the *Euryarcha-*

eota (Fig. 2) phylum, and were phylogenetically related to sequences which had been recovered from other marine and methane-rich environments (Brehmer, unpublished; Munson *et al.*, 1997; Vetriani *et al.*, 1998, 1999; Hinrichs *et al.*, 1999; van der Maarel *et al.*, 1999; Teske *et al.*, 2002; Inagaki *et al.*, 2003). Clone BS1-1-84 was similar to a methanogen, *Methanococcoides methylutens* (98.6% similarity). The latter was isolated from marine sediment, and was demonstrated to grow only on methylamines, degradation products of the algal osmolyte glycine betaine, or methanol (van der Maarel *et al.*, 1999). It was shown that these substrates can not be utilized by sulfate-reducing bacteria, and are thus considered 'non-competitive' substrates, which are used exclusively by methanogens (Oremland and Polcin, 1982). Sulfate-reducing bacteria were found to be abundant in tidal flat sediment (Kim *et al.*, 2004). As is frequently observed in the 16S rRNA-based surveys of natural samples (DeLong, 1992; Fuhrman *et al.*, 1992; McInerney *et al.*, 1995), none of these sequences were identical to the reference sequences from cultured taxa, although some were found to be closely related to sequences from cultured methanogens that had been previously isolated from marine sediments. Clone BS1-1-14, for instance, was clustered with the cultured methanogen group (90.63% similarity to *Methanococcoides* sp. NaT1), but was only distantly related to other sequences, and represented a deep branch within this group. Clone BS1-1-64 was found to be similar to the thermophilic acetoclastic methanogen, *Methanosarcina thermophila* (GenBank # M59140), but represented a deep branch within the *Methanosarcinales* cluster, which was clustered with the O23F7 clone from the Monterey Formation reservoir (Orphan *et al.*, 2000). Two clones (BS1-1-47, -99) were determined to be closely related to the SYA 2000-35 clone obtained from Lake Soyang in South Korea (83.69 and 96.3% similarity, GenBank # AF291787). The uncultured euryarchaeal clone sequences obtained from the getbol were found to be relatively similar to one another, and were clustered with the sequences obtained from other marine sediments, lakes, gas hydrates, and gold mine waters.

Discussion

The identification of *Archaea* from tidal flat sediment is a crucial step in our understanding of the ecological significance of *Archaea* in the biosphere, and is also important to our analysis of naturally-occurring microbial communities. Thus far, a large number of novel archaeal phylotypes have been located, in a variety of microbial habitats, including those in open ocean waters (Fuhrman *et al.*, 1992), coastal waters (DeLong, 1992; Preston *et al.*, 1996; Massana *et al.*, 1997), polar seas (Vetriani *et al.*, 1998, 1999; Reysenbach *et al.*, 2000), salt marshes (Munson *et al.*, 1997), freshwater lakes (Go and Ahn, unpub-

lished; Hershberger et al., 1996; Jurgens et al., 2000), agricultural and forest soils, including the rhizosphere (Borneman and Triplett, 1997; Buckley et al., 1998), paddy field soil (Gro kopf et al., 1998; Chin et al., 1999), hot springs (Barns et al., 1994, 1996), deep-sea hydrothermal vents (Takai and Horikoshi, 1999a), mine water (Takai et al., 2001) and deep subsurface geothermal pools (Takai and Horikoshi, 1999b). The phylogenetic diversity of Archaea has been extended substantially by these investigations. In addition, comparative phylogenetic analyses of environmental archaeal clones have revealed many of the characteristic phylogenetic features of the archaeal community. In order to evaluate the diversity of Archaea in the sediments of tidal flat, we obtained total DNA directly from sediment, and the archaeal rDNA sequences were selectively amplified by PCR, and then cloned. A total of 102 tidal flat sediment clones were sequenced and analyzed, via comprehensive phylogenetic analysis. Based on thorough analyses, these clones were primarily related to the sequences obtained for a variety of marine environmental clones. Sequences that are closely related to our crenarchaeal clones have been found in marine sediments from Japan, which is located near Korea (Reed et al., 2002; Inagaki et al., 2003).

Although physiology cannot necessarily be assumed from phylogeny, in many cases the physiology of an organism can be cautiously inferred from its phylogeny. If a sequence is similar to that of a group of cultivated organisms with common properties, then the environmental organism represented only by the sequence should also be expected to exhibit those properties. For instance, methanogens are clearly involved in the complete remineralization of sedimentary organic matter to methane, and are commonly associated with anoxic marine sediments (Vetriani et al., 1998). The close affiliation of euryarchaeal clones to the methylotrophic methanogen, M. methylutens, suggests that methanogens found in the intestines of fish and suspended particulate matter are also methylotrophs, and thus are probably also capable of converting methanol or methylamines (van der Maarel et al., 1999). The microbial communities that inhabit marine sediments are important for biological carbon and sulfur cycling (Nedwell, 1984). The crenarchaeal clones in our library were found to be clustered with sulfur-reducing heterotrophs, and most of the bacterial clones from the previous study appeared to represent sulfur-reducing bacteria (Kim et al., 2004). In marine sediments, the final steps in the mineralization of organic carbon to CH_4 or CO_2 are believed to be carried out by either methanogenic archaea or sulfate-reducing bacteria. When sulfates are freely available (e.g., in marine sediments), sulfate-reducing bacteria outcompete methanogenic archaea. This is due to the fact that cultured strains exhibit higher specific affinity for acetates and H_2, which are utilized by both groups (Lovely et al., 1982). The archaeal populations in

this study have been associated with both carbon and sulfur cycling in tidal flat sediments.

When discussing 16S rDNA sequence data, it is important to note that the diversity suggested by the data is not necessarily representative of the in situ community of microorganisms in the sediments. Rather, it tends to be representative of the libraries which have been constructed (von Wintzingerode et al., 1997). However, in spite of these reservations, it is clear that molecular ecological methods have tended to reveal a level of diversity which is far greater than that associated with any previously isolated microorganisms. If a bias does exist in these methods, examinations of a variety of environments would suggest that it is a consistent bias, and as these methods improve, we should begin to see previously unrecognized biases or artifacts (Inagaki et al., 2003).

The archaeal communities observed in clone libraries obtained from tidal flat sediment are quite unlike the archaeal communities obtained from other marine sediments (Munson et al., 1997; Vetriani et al., 1999), in that the diversity of the tidal flat sediment archaea tends to be much higher, and in that the sequences obtained from the tidal flat sediment clones are closely related to the sequences obtained from various environmental clones by comparative phylogenetic analysis. For instance, our Crenarchaeota clones were clustered with clones from hydrothermal vents, anoxic sediments, and low-temperature freshwater. The Euryarchaeota clones were involved with methanogenic clones, which are usually associated with both low-temperature anoxic marine sediments and lake water clones. This may be attributable to the fact that tidal flats maintain a dynamic condition, including periodic flooding and exposure to seawater, which provides an extra set of environmental physiochemical variables. In addition, soil particle size fractions (sand, silt, clay) were associated with distinct microbial community structures, and particle size was one factor which influenced the structure of microbial communities (Sessitsch et al., 2001). Watts (1999) found that some variation existed in the structure of the bacterial community between different soil aggregate size classes, ranging from $\geq$ 250 to < 1 μm; actinomycetes were most abundant in the larger aggregates, whereas pseudomonads were most abundant in the smaller aggregates. The soil of tidal flat sediments was characterized by variable particle size (4% of clay, 55.4% of silt and 40.6% of sand), where the particle size of the clay was 0.2 μm - 2 μm, the silt was 2 μm - 40 μm, and the particle size of sand was 20 μm - 2 mm (Klute, 1986). Archaeal clones exhibited a higher similarity to the 16S rDNA clone sequences from other environments in the public database than that of bacterial clones previously reported bacterial clones in tidal flat sediment (Kim et al., 2004). This is likely attributable to the fact that Bacteria are more abundant and more diverse than Archaea in tidal flat sediment.

The 16S rDNA sequences determined in this study expand our current knowledge regarding the archaeal community in tidal flat sediment, and provide a framework for future molecular ecological studies, using relatively high-throughput techniques, such as denaturing gradient gel electrophoresis. In addition, biogeochemical measurements will constitute a critical next step in the correlation of the distribution of novel *Archaea* with their potential activity and ecological roles in tidal flat sediments.

Acknowledgements

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