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Remarkable Bacterial Diversity in the Tidal Flat Sediment as Revealed by 16S rDNA Analysis

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Abstract A 16S rDNA clone library was generated to investigate the bacterial diversity in tidal flat sediment in Ganghwa Island, Republic of Korea. A total of 103 clones were sequenced and analyzed by comprehensive phylogenetic analyses. No clones were identical to any of known 16S rRNA sequences in public databases. Sequenced clones fell into thirteen lineages of the domain *Bacteria*: the alpha, beta, gamma, delta, and epsilon *Proteobacteria*, *Actinobacteria*, CFB group, *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Verrucomicrobia*, and known uncultured candidate divisions (OP11, BRC1, KSB1, and WS1). Two clones were not associated with any known bacterial divisions. The majority of clones belonged to the gamma and delta *Proteobacteria* (46.7%). Clones of *Actinobacteria* were distantly related to known taxa. It is evident from 16S rDNA-based community analysis that the bacterial community in tidal flat sediment is remarkably diverse and unique among other marine environments examined so far.

Key words: Tidal flat, 16S rDNA sequencing, diversity, phylogeny

Microbes in marine sediments are responsible for various important biochemical transformations, including the mineralization of organic matter and the degradation of pollutants. In recent years, pollution-induced changes have been recognized in the benthic microbial community structure [26]. To better understand these processes, a better understanding of the structure of the microbiota involved is needed. However, defining or quantitatively describing the microbial community structure is often difficult and incomplete. In addition, both sediment and soil probably represent some of the most complex microbial habitats on earth: a single gram of soil may contain thousands of bacterial species [38]. One of the important steps in studying the microbial community in marine sediments is to clarify how the physical and chemical properties of the sediments affect the whole community structure. However, many studies of microbes in marine sediments have focused on specific functional groups, because the processes they catalyze can be measured directly [18, 34]. In general, the depth-related gradient of physical and chemical properties provides niches for a wide variety of metabolically diverse microorganisms in marine sediments. In such environments, syntrophic and competitive interactions occur between different physiological types of microorganisms. However, few studies have been carried out to provide an overview of the microbial population in marine sediments and how this is distributed in relation to the chemical environment and associated processes [25].

The west and southwest coasts of the Korean peninsula largely consist of tidal flats, called getbol. Getbol can be several kilometers long when the tide is high, and plays an important role in the Korean coastal fishery industry. The environment is unique among other marine sediments as it is flooded and exposed periodically by the seawater. The higher degree of changes in water temperature and salinity are often observed. Getbol has been shown to be one of the most dynamic areas in terms of sediment erosion and deposition, and it can be distinguished from salt marsh and wetland for these characteristics [2].

To understand the biological and ecological role of getbol, it is important to elucidate its microbial components. The analysis of the composition of natural microbial populations has a long tradition in microbial ecology. Classical cultivation-based techniques are insufficient for studying the diversity of naturally occurring microbial communities since the majority of microbial organisms is believed to be uncultivable by traditional techniques [1]. Several molecular approaches now provide powerful means

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to culture-dependent techniques [21, 24]. As a basic approach to clarifying microbial communities, 16S rDNAs are amplified by PCR from nucleic acids extracted from environmental samples, and then the PCR products are cloned and sequenced. This approach can avoid the limitations of the traditional culturing techniques for assessing the microbial diversity in natural environments [39].

There have been only a few studies investigating bacterial community in marine sediments using culture-independent methods [9, 12, 39]. Furthermore, there is no report to examine microbial community structure in extensive tidal flats such as getbol in Korea. In a recent study, a large number of novel bacterial species have been isolated, where 20–30% were found to be novel species, using standard isolation technique [41]. This remarkable cultured bacterial diversity led to the study of its uncultured microbial community. The aim of this study is to investigate the diversity of the bacterial community in Korean getbol sediments, which will help with understanding its role in bioremediation and material cycling. This information can be a framework for future 16S rDNA-based community analysis.

Getbol sediments were collected from a site at Dongmak (37°35.319'N, 126°27.245'E) in Ganghwa Island. Sections from depths of 5 cm were subsampled and placed in polypropylene bags. Samples were immediately stored on dry ice for transport to the laboratory. The samples were stored at -80°C until DNA was extracted.

Soil pH was determined with 10:1 soil slurry, organic matter content was measured by the loss-on-ignition method, and soil texture was determined as previously described [29]. Chemical properties of the pore-water were measured after extracting sediment with deionized water. Dissolved organic carbon (DOC) content was measured by a TOC meter (Shimadzu-5000A) and the concentration of NO_3^- was determined by ion chromatography (Dionex DX-120).

DNAs from 10 g of soil were extracted by the CsCl density equilibrium gradient methods according to the Direct lysis protocol for Bacterial community DNA Recovery (CsCl) [17]. PCR amplification of bacterial 16S rDNA from getbol DNA extracts was performed with a total volume of 50 µl in a thermal cycler. Bacterial 16S rDNAs were enzymatically amplified with primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGYTACCTTGTTACGACTT-3') as described [4].

PCR products were purified with a PCR Clean-up Kit (Mo Bio). 16S rDNA amplicons were ligated into the pGEM-T easy vector (Promega) and transformed into *E. coli* DH10B cells. Colony PCR was performed as above with the following modifications [3, 31]. The primers used were prGTf (5'-TACGACTCACTATAGGGCGA-3') and prGTr (5'-CTCAAGCTATGCATCCAACGC-3') targeting the flanking regions of the multicloning site of pGemT-easy vector [5].

To examine the ARDRA patterns, 1 µl of the colony PCR product was digested with two four-base-specific restriction enzymes (*Hha*I, *Hae*III) (NEB) at 37°C for 2 h. The resultant fragments were analyzed by electrophoresis in 2% agarose gels (APB). 100 bp ladder (APB) was used as a DNA marker.

PCR products were cleaned up using shrimp alkaline phosphatase and the *Exo*I treatment method following ABI PRISM SnaPshot Multiplex Kit Protocol. Sequencing was achieved using an automated DNA sequencer (ABI Gene Scan 3100). Eight clones (BS1-0-27, 34, 55, 74, 79, 85, 86, 111) were sequenced in complete length to further clarify their phylogenetic positions. All sequences were checked for chimeric artifacts by the CHECK_CHIMERA program of the Ribosomal Database Project (RDP) [27], and manually aligned with closely related sequences obtained from the Ribosomal Database Project and GenBank databases. The calculation of sequence similarity and phylogenetic tree inference were carried out using the PHYDIT program (available at http://plaza.snu.ac.kr/~jchun/phydit/). An evolutionary tree was generated using the neighbor-joining method [36]. Evolutionary distance matrices were generated according to Jukes and Cantor [19]. The resultant tree topologies were evaluated in bootstrap analyses [10] based on 1,000 resamplings.

The GenBank accession numbers for the sequences determined in this study are AY254909-24, AY254926-45, AY254947-48, AY254950-70, AY254972-76, AY254978-94, AY254996-97, AY254999-5003, and AY304363-77.

The pH, organic matter (%), DOC (mg/l^{-1}), and NO^{3-} (mg/l^{-1}) of the getbol sediments sample were 6.77, 2.2, 5.7, and 1.13, respectively. The soil texture was clay 4%, silt 55.4%, and sand 40.6%.

Nucleic acids were successfully extracted from the getbol sediments (data not shown) without polyvinyl polypyrrolidone purification, which has been used for extracting DNA from humic acid-rich samples [14] for PCR amplification. The CsCl density equilibrium gradient methods used in this study were successful in obtaining high molecular weight DNA from getbol. The procedures may be applicable to a variety of bacterial communities in nature. Extracted DNA was loaded onto 1% agarose gel. DNAs were amplified with the PCR.

The ARDRA patterns were used as an initial measure of diversity in the getbol. However, clones were so diverse that most of them were not clustered by the ARDRA patterns (data not shown). All clones were therefore examined by sequencing methods.

The average length of the sequences determined was 650 bases. Using the RDP' CHECK_CHIMERA, 7 clones were found to be probable chimeric amplicons and omitted from the final analysis.

Thirteen major phylogenetic bacterial lineages were recovered from the getbol sediment: α-*Proteobacteria* (13

Fig. 1. Phylogenetic tree based on 16S rDNA sequences of the proteobacterial clones from tidal flat sediment. The tree was constructed using the neighbor-joining method.

Bacillus subtilis was used as the outgroup. The percentage numbers at the nodes are the levels of bootstrap support based on neighbor-joining analysis of 1,000 resampled data sets. Bar, 10% nucleotide changes per a 16S rDNA position.

clones), β-*Proteobacteria* (1), δ-*Proteobacteria* (20), γ-*Proteobacteria* (28), ε-*Proteobacteria* (2), *Actinobacteria* (3), *Cytophaga-Flavobacterium-Bacteroides* (CFB) group (11), *Acidobacteria* (5), *Planctomycetes* (2), *Verrucomicrobia* (2), *Chloroflexi* (8), and uncultured candidate divisions OP11 (1), BRC1 (1), KSB1 (2), and WS3 (2). Two clones showed no clear association to any known bacteria divisions.

Phylogenetic analysis showed that γ-*Proteobacteria* was the major component of tidal flat sediment (27.2% of total clones). Similar results were reported for samples collected from arctic ocean sediment [33] and marine sediments in Washington, USA, and Japan [12, 39]. The phylogenetic positions of 28 γ-proteobacterial clones together with other proteobacteria are given in Fig. 1. The tree included sequences isolated in other marine sediments [6, 7, 13, 22, 23, 34, 41]. Three clones (BS1-0-25, 65, and 112) formed a monophyletic clade with Norwegian cold marine sediment clone Sva0091 (GenBank # AJ240987) and represented a deep branch within γ-*Proteobacteria*. Similarly, twelve unique clones (BS1-0-3, 21, 28, 38, 60, 84, 92, 102, 106, 107, 110, and 117) were recovered in a monophyletic clade containing marine sediment clones in the Japanese Trenches. Bacteria belonging to this clade may be widely spread in the Korea-Japan area through surface and deep-sea sediments, though there are no cultured representatives in this group yet. The highest similarity value in the γ-*Proteobacteria* was found between clone BS1-0-84 and Japan Trench sediment clone NB1-h (96.36% similarity) [40].

The second most abundant group was δ-*Proteobacteria* (19.5%) which was reported to be also a predominant group in various marine sediments throughout the world [6, 12, 34, 40]. Most of the clones were associated with sulfate-reducing bacteria. Four clones (BS1-0-16, 58, 98, and 104) formed a monophyletic clade and were distantly related to other cultured and uncultured sulfate-reducing bacteria. This group clearly represents a novel sulfate-reducing bacterial lineage in the test sample. Occurrence of sulfate-reducing microorganisms in tidal flat sediment is not a surprise as sulfate is one of the main electron acceptors present in marine environments. In addition, sulfate-reducing bacteria are able to utilize a variety of electron donors available in marine sediments, such as carbon compounds released by plant roots or fermentation products of other bacteria including acetate, lactate, butanol, and formate [36]. A number of studies showed that metabolically diverse species of sulfate-reducers were present in various marine sediments [6, 12, 32, 33, 39].

Thirteen clones belonged to α-*Proteobacteria* (Fig. 1) and were clustered with cultured marine-dwelling bacteria such as *Roseobacter*, *Sulfitobacter*, and *Lutibacterium*. Only one and two clones were found to be members of β-*Proteobacteria* and ε-*Proteobacteria*, respectively (Fig. 1). The β-proteobacterial clone (BS1-0-10) was most similar to *Nitrosomonas* strain Nm143 (GenBank # AY123794) and type strain of *Nitrosomonas europaea*, but the corresponding 16S rDNA similarities were very low (91.8–89.9%). One of ε-*Proteobacteria* clone (BS1-0-114) was clustered with the Nankai Trough sediment clone at 3,843 m with 96.8% similarity. The other clone (BS-1-0-67) formed a novel phyletic lineage and its closest relative was a clone (33-PA23B00) from deep-sea sediment with volcanic eruption [15], with very low similarity of 77.5%.

Five clones (BS1-0-2, 93, 105, 116, 123) were found within *Acidobacteria* (Fig. 2A). Clones in this group were clustered with Norwegian cold marine sediments (Sva0515, GenBank # AJ241004 and Sva0450, GenBank # AJ240998 [33]) and hydrocarbon seep sediment clone BPC102 (GenBank # AF154083). Two clones (BS1-0-53 and 88) were recovered in *Planctomycetes* (Fig. 2A). The closest neighbor to these clones was German river biofilm clone DSP26 (GenBank # AJ290184; 86.9–92.3% similarity).

Three clones (BS1-0-27, 74, 79) in *Actinobacteria* were closely related to uncultured actinobacteria clone (Fig. 2B). These clones were fully sequenced, and their phylogenetic relationship was established. These clones, together with Japan Suruga Bay deep-sea sediment clone BD2-10 (GenBank # AB015539) [22] and marine sediment clone (GenBank # AY193208), formed a clade outside to cultured actinomycetes. Sequence similarity values within this clade were lower than 84%, which indicates that many novel phyletic actinomycete lineages can be found in marine sediment environments.

Two clones were associated with the *Verrucomicrobia* group (Fig. 2C). BS1-0-101 was closest to Norwegian cold marine sediments clone Sva0700 (GenBank # AJ297462) [33].

Six clones belonged to candidate divisions that do not have cultured representatives so far (Fig. 2C). Two clones (BS1-0-32 and 109) were members of the candidate division WS3 [11]. Both clones showed high, but distant, similarity with anoxic marine sediment clone LD1-PA30 (GenBank # AY114319) with 84.0% for BS1-0-32 and 93.4% for BS1-0-109, respectively. The similarity value between the two getbol clones was 84.6%. Clone BS1-0-111 belonged to the candidate division OP11, being closely related to Antarctica continental sediment clone MERTZ 21CM (GenBank # AF424451) with 95.6% similarity. Two clones (BS1-0-24 and 55) were members of the candidate division KSB1 [38], whereas clone BS1-0-85 was related to the bulk soil clone PBS-III-24 of the candidate division BRC1 (GenBank # AJ390454) [8]. Clone BS1-0-11 was closely related to a hitherto-unknown division represented by clone mle1-16 from pharmaceutical wastewater [20] with a similarity of 94.5%. Similarly, clone BS1-0-119, together with clone BD2-6 from Japan Suruga Bay 1521 m sediment [22], formed a hitherto unknown division. The 16S rDNA similarity between clones BS1-0-119 and BD2-6 was 92.2%.

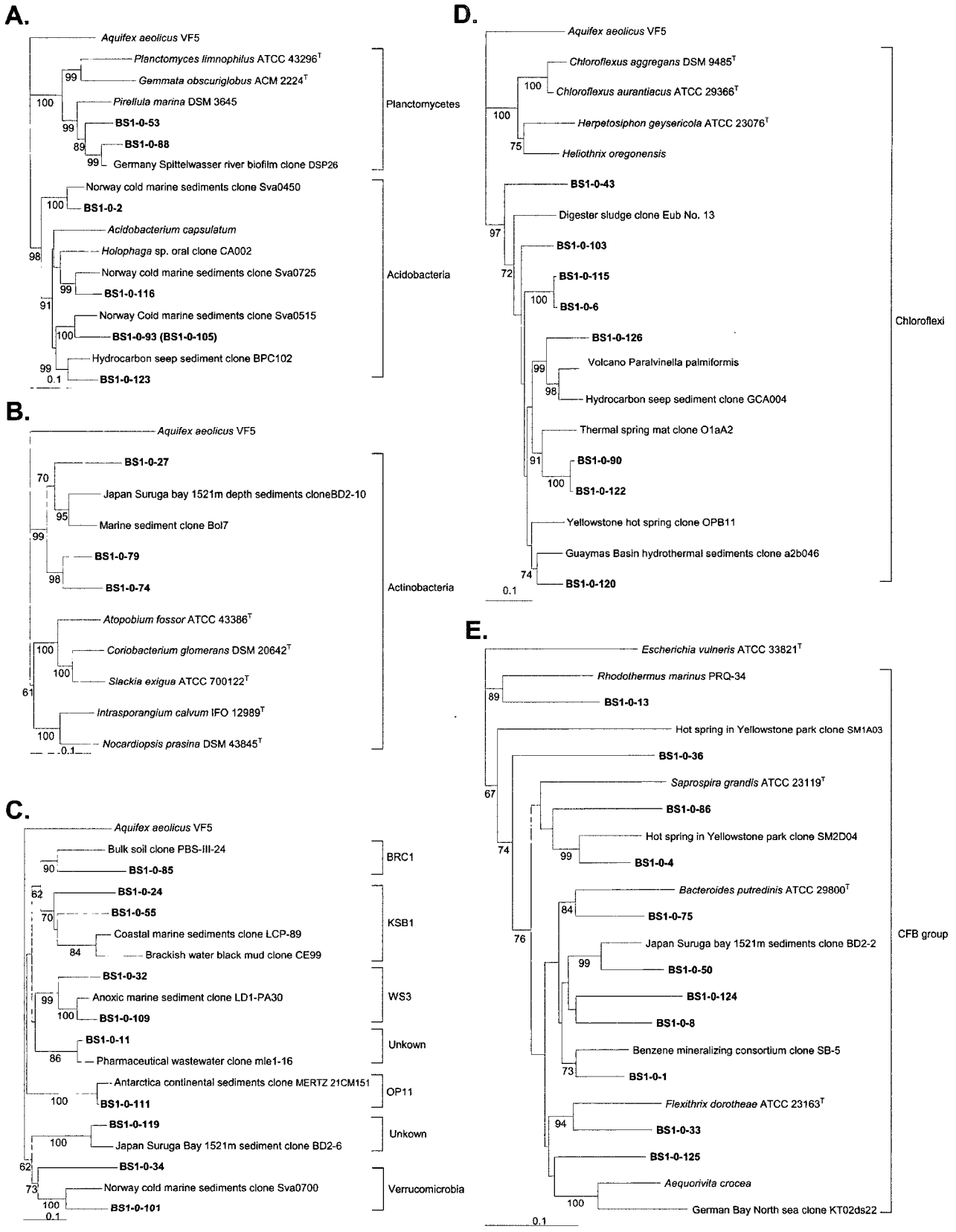

Fig. 2. Phylogenetic trees of the *Planctomycetes* and *Acidobacteria* (A), *Actinobacteria* (B), candidate divisions and *Verrucomicrobia* (C), *Chloroflexi* (D), and CFB group (E). Bar, 10% nucleotide changes per a 16S rDNA position.

Eight clones were affiliated with *Chloroflexi* (Fig. 2D). Clones in this group were clustered with hydrocarbon seep sediment clone GCA004 (GenBank # AF154104) and Axial Volcano *Paralvinella palmiformis* mucus secretions clone P. palm C 37 (GenBank # AJ441227) [16].

Phylogenetically diverse CFB clones were recovered from a tidal flat sediment sample (Fig. 2E). Among the eleven clones, the highest similarity value to known sequences in GenBank was only 89.8% for clone BS1-0-1 and benzene mineralizing consortium clone SB-5 (GenBank # AF029041) [31]. The lowest similarity (77.9%) was found between clone BS1-0-118 and Norwegian cold marine sediment clone Sva0515 (GenBank # AJ214004). The very low similarity values and branching patterns in the phylogenetic tree indicate that bacteria belonging to the CFB group in tidal flat sediment are extremely diverse and unique.

The average sequence similarity of tidal flat sediment clones to known sequences in GenBank was only 88.4%, ranging from 74.9 to 97.6%. It is clear from these low values and diverse lineages found in the phylogenetic tree analysis that bacterial diversity in the test sample is remarkably higher than those in most of the other marine sediments [28, 39]. This is likely due to the tidal flat being flooded and exposed periodically by the seawater, which adds extra physiochemical variables to the environments. In conclusion, the 16S rDNA sequences determined in this study expand the knowledge of the bacterial community in marine tidal flat sediment and provide a firm framework for future molecular ecological studies using relatively high-throughput techniques such as denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism.

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REFERENCES

1. Amann, R. I., W. Ludwig, and K. H. Schleifer. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol. Rev.* **59:** 143-169.
2. Carling, P. A. 1982. Temporal and spatial variation in intertidal sedimentation rates. *Sedimentol.* **29:** 17-23.
3. Choi, H.-P., H.-J. Kang, H.-C. Seo, and H.-C. Sung. 2002. Isolation and identification of photosynthetic bacterium useful for waste water treatment. *J. Microbiol. Biotechnol.* **12:** 643-648.
4. Chun, J. and M. Goodfellow. 1995. A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int. J. Syst. Bacteriol.* **45:** 240-245.
5. Chun, J., A. Huq, and R. R. Colwell. 1999. Analysis of 16S-23S rRNA intergenic spacer regions of *Vibrio cholerae* and *Vibrio mimicus*. *Appl. Environ. Microbiol.* **65:** 2202-2208.
6. Cifuentes, A., J. Anton, S. Benlloch, A. Donnelly, R. A. Herbert, and F. Rodriguez-Valera. 2000. Prokaryotic diversity in *Zostera noltii*-colonized marine sediments. *Appl. Environ. Microbiol.* **66:** 1715-1719.
7. Crump, B. C., E. V. Armbrust, and J. A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the columbia river, its estuary, and the adjacent coastal ocean. *Appl. Environ. Microbiol.* **65:** 3192-3204.
8. Derakshani, M., T. Lukow, and W. Liesack. 2001. Novel bacterial lineages at the (sub)division level as detected by signature nucleotide-targeted recovery of 16S rRNA genes from bulk soil and rice roots of flooded rice microcosms. *Appl. Environ. Microbiol.* **67:** 623-631.
9. Devereux, R., M. Delaney, F. Widdel, and D. A. Stahl. 1989. Natural relationships among sulfate-reducing eubacteria. *J. Bacteriol.* **171:** 6689-6695.
10. Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783-791.
11. Freitag, T. E. and J. I. Prosser. 2003. Community structure of ammonia-oxidizing bacteria within anoxic marine sediments. *Appl. Environ. Microbiol.* **69:** 1359-1371.
12. Gray, J. P. and R. P. Herwig. 1996. Phylogenetic analysis of the bacterial communities in marine sediments. *Appl. Environ. Microbiol.* **62:** 4049-4059.
13. Hedlund, B. P., A. D. Geiselbrecht, T. J. Bair, and J. T. Staley. 1999. Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov. *Appl. Environ. Microbiol.* **65:** 251-259.
14. Holben, W. E., J. K. Jansso, B. K. Chelm, and J. M. Tiedje. 1988. DNA probe method for the detection of specific microorganisms in the soil bacterial community. *Appl. Environ. Microbiol.* **54:** 703-711.
15. Huber, J. A., D. A. Butterfield, and J. A. Baross. 2003. Bacterial diversity in a subseafloor habitat following a deep-sea volcanic eruption. *FEMS Microbiol. Ecol.* **43:** 393-409.
16. Hugenholtz, P., C. Pitulle, K. L. Hershberger, and N. R. Pace. 1998. Novel division level bacterial diversity in a Yellowstone hot spring. *J. Bacteriol.* **180:** 366-376.
17. Hurst, C. J. 1997. Recovery of bacterial community DNA from soil, pp. 433-434. *In* Hurst, C. J., R. L. Crawford, G. R. Knudsen, M. J. McInerney and L. D. Stetzenbach (eds.), *Manual of Environmental Microbiology*, 2nd ed. ASM Press, Washington DC, U.S.A.
18. Jorgensen, B. B. 1982. Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **298:** 543-561.
19. Jukes, T. H. and C. R. Cantor. 1969. Evolution of protein molecules, pp. 21-132. *In* Munro, H. N. (ed.), *Mammalian Protein Metabolism*. Academic Press, New York, U.S.A.
20. LaPara, T. M., C. H. Nakatsu, L. Pantea, and J. E. Alleman. 2000. Phylogenetic analysis of bacterial communities in mesophilic and thermophilic bioreactors treating pharmaceutical wastewater. *Appl. Environ. Microbiol.* **66:** 3951-3959.

21. Lee, W. J. and K. S. Bae. 2001. The phylogenetic relationship of several oscillatorian cyanobacteria, forming blooms at Daecheong reservoirs, based on partial 16S rRNA gene sequences. *J. Microbiol. Biotechnol.* **11:** 504-507.
22. Li, L., C. Kato, and K. Horikoshi. 1999. Bacterial diversity in deep-sea sediments from different depths. *Biodivers. Conserv.* **8:** 659-677.
23. Li, L., C. Kato, and K. Horikoshi. 1999. Microbial diversity in sediments collected from the deepest cold-seep area, the Japan Trench. *Mar. Biotechnol.* **1:** 391-400.
24. Liu, W. T., T. L. Marsh, H. Cheng, and L. J. Forney. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl. Environ. Microbiol.* **63:** 4516-4522.
25. Llobet-Brossa, E., R. Rossello-Mora, and R. Amann. 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence *in situ* hybridization. *Appl. Environ. Microbiol.* **64:** 2691-2696.
26. MacNaughton, S. J., J. R. Stephen, A. D. Venosa, G. A. Davis, Y. J. Chang, and D. C. White. 1999. Microbial population changes during bioremediation of an experimental oil spill. *Appl. Environ. Microbiol.* **65:** 3566-3574.
27. Maidak, B. L., G. J. Olsen, N. Larsen, R. Overbeek, M. J. McCaughey, and C. R. Woese. 1997. The RDP (Ribosomal Database Project). *Nucleic Acids Res.* **25:** 109-111.
28. Mullins, T. D., T. B. Britschgi, R. L. Krest, and S. J. Giovannoni. 1995. Genetic comparisons reveal the same unknown bacterial lineages in atlantic and pacific bacterioplankton communities. *Limnol. Oceanogr.* **40:** 148-158.
29. Page, A. L., R. H. Miller, and D. R. Keeney. 1982. *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*, 2nd ed. American Society of Agronomy, Madison, WI, U.S.A.
30. Park, J., B., H. Lee, W., S.-Y. Lee, J. O. Lee, I. S. Bang, E. S. Choi, D. H. Park, and Y. K. Park. 2002. Microbial community analysis of 5-stage biological nutrient removal process with step feed system. *J. Microbiol. Biotechnol.* **12:** 929-935.
31. Phelps, C. D., L. J. Kerkhof, and L. Y. Young. 1998. Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. *FEMS Microbiol. Ecol.* **27:** 269-279.
32. Ravenschlag, K., K. Sahm, C. Knoblauch, B. B. Jorgensen, and R. Amann. 2000. Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine arctic sediments. *Appl. Environ. Microbiol.* **66:** 3592-3602.
33. Ravenschlag, K., K. Sahm, J. Pernthaler, and R. Amann. 1999. High bacterial diversity in permanently cold marine sediments. *Appl. Environ. Microbiol.* **65:** 3982-3989.
34. Sahm, K., C. Knoblauch, and R. Amann. 1999. Phylogenetic affiliation and quantification of psychrophilic sulfate-reducing isolates in marine arctic sediments. *Appl. Environ. Microbiol.* **65:** 3976-3981.
35. Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4:** 406-425.
36. Smith, W. 1993. Ecological actions of sulfate-reducing bacteria, pp. 161-188. *In* Odom, J. M. and R. Singleton (eds.), *The Sulfate-Reducing Bacteria: Contemporary Perspectives.* Springer-Verlag, New York, U.S.A.
37. Tanner, M. A., C. L. Everett, W. J. Coleman, M. M. Yang, and D. C. Youvan. 2000. Complex microbial consortia inhabiting hydrogen sulfide-rich black mud from marine coastal environments. *Biotechnol. et alia* **8:** 1-16.
38. Torsvik, V., J. Goksoyr, and F. L. Daae. 1990. High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* **56:** 782-787.
39. Urakawa, H., K. Kita-Tsukamoto, and K. Ohwada. 1999. Microbial diversity in marine sediments from Sagami Bay and Tokyo Bay, Japan, as determined by 16S rRNA gene analysis. *Microbiology* **145:** 3305-3315.
40. Yanagibayashi, M., Y. Nogi, L. Li, and C. Kato. 1999. Changes in the microbial community in Japan Trench sediment from a depth of 6292 m during cultivation without decompression. *FEMS Microbiol. Lett.* **170:** 271-279.
41. Yi, H. and J. Chun. 2002. Remarkable cultured bacterial biodiversity in getbol, the tidal flat of Korea. IUMS World Congress, Paris, France.