Phylogenetic diversity and distribution of dissimilatory sulfite reductase genes from deep-sea sediment cores

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Running title: Deep-sea dissimilatory sulfite reductase genes

Abstract

The diversity and distribution of sulfate-reducing prokaryotes (SRP) was investigated in the Nankai Trough sediments of off-central Japan by exploring the diversity of a functional gene, dissimilatory sulfite reductase (*dsrAB*). Bulk DNAs were extracted from five piston-cored samples (up to 4.5 m long) with 41 vertical sections, and full-length *dsrAB* gene sequences (*ca*. 1.9 kb) were PCR amplified and cloned. A total of 382 *dsrAB* clones yielded eight phylogenetic groups with an indigenous group forming a unique *dsrAB* lineage. The deltaproteobacterial *dsrAB* genes were found in almost all sediment samples, especially in the surface layer. One unique *dsrAB* clone group was also widespread in the *dsrAB* profiles of the studied sediments, and the percentage of its clones was generally shown gradual increase with sediment depth.

Key words: sulfate reduction, sulfate-reducing prokaryotes, Nankai Trough.

Introduction

Sulfate-reducing prokaryotes (SRP) are key and ubiquitous mediators of global sulfur cycling, particularly in marine sediments where sulfate serves as a major anaerobic oxidant (Jørgensen, 1977; Jørgensen, 1982). As culture-dependent technique may miss dominant and/or key environmental species (Amann et al., 1995; Suzuki et al., 1997), 16S rRNA gene-based molecular approaches such as PCR clone library analyses and hybridizations including FISH using taxon-specific probes have been applied to survey

distribution and diversity of SRP in marine sediments (e.g. Devereux and Mundfrom, 1994; Llobet-Brossa et al., 1998; Bowman and McCuaig, 2003). Another molecular approach aims at the genes, *dsrAB*, encoding the subunits of dissimilatory sulfite reductase (EC 1.8.99.3) (Molitor et al., 1998; Hatchikian and Zeikus, 1983). The *dsrAB* genes are unique to SRP (Stahl et al., 2002) and have been used as diagnostic and phylogenetic tools to study distribution and diversity of SRP (e.g. Fukuba et al., 2003; Loy et al., 2004; Nakagawa et al., 2004; Pérez-Jiménez et al., 2005).

SRP in top <1 m sediments have mainly been studied (e.g. Devereux and Mundfrom, 1994; Mußmann et al., 2005; Ravenschlag et al., 2000; Purdy et al., 2003); however, sulfate reduction occurs even in a few to tens m below seafloor as revealed by the Ocean Drilling Program (D'Hondt et al., 2002; D'Hondt et al., 2004; Mauclaire et al., 2004; Schippers and Neretin, 2006). One of the few studies targeting the sediment several m below seafloor suggested the occurrence of novel SRP possessing deeply-branching *dsrAB* (Thomsen et al. 2001), which may be missed by using taxon-specific 16S rRNA gene primers and probes. We have thus investigated distribution and diversity of *dsrAB* in <4.5-m-thick sediment cores collected from the Nankai Trough seafloor, off central Japan, where a number of cold-seep fauna have been studied (Li et al., 1999; Arakawa et al., 2006).

Materials and Methods

Five sediment cores containing silt, clayey silt and very fine sand were collected from three areas in the Nankai Trough, off central Japan (Table 1), using a 5 m-long piston corer with a dispensable acryl inner

tube. Interstitial sulfate concentration decreased with depth from 30 mM (top) to 13 mM (4.3 m below seafloor), suggesting that cored depths were shallower than the sulfate-methane interface (Tanahashi et al., unpublished). The core tubes were longitudinally halved, and a total of 41 samples from different sediment layers were collected using sterile 5-ml syringes modified by removal of end flanges to form open cylinders (Terumo, Tokyo, Japan). The mud-filled syringes were plugged and frozen at -80^{o}C onboard.

Bulk DNAs were extracted from 200 mg of each of the thawed samples by bead-beating (Miller et al., 1999) with 2 g each of 0.1-mm zirconium silica beads in 0.3 ml of phosphate buffer (100 mM NaH_2PO_4; pH 8.0), 0.3 ml of lysis buffer (10% SDS, 100 mM NaCl, and 500 mM Tris; pH 8.0) and 0.3 ml of chloroform-isoamyl alcohol (24:1) in 2-ml tubes. A Mini Bead Beater-8 (BioSpec Product, Bartlesville, Oklahoma) was used at 3200 rpm for 4 min to lyse cells, and 0.1 ml of each of the supernatants was filter-purified with a Chroma Spin+TE1000 Column (BD Biosciences, San Jose, California) after centrifugation at 21600 x *g* for 5 min. The bulk DNAs in the filtrates were amplified using the GenomiPhi DNA Amplification Kit (GE Healthcare, Buckinhamshire, UK) to secure the amount of source DNA for repeated trials, and then used as PCR templates for *dsrAB*. Whole genome amplification by multiple displacement amplification (MDA) is probably the only technique to start a study with only trace amounts of DNA (*e.g.* Gonzalez et al., 2005). However, quantitative value of the study may be damaged by the biases introduced by MDA (Abulencia et al., 2006). On the other hand, MDA with ϕ29 DNA polymerase may result in low-biased amplification (Dean et al., 2002; Hosono et al., 2003; Luthra and Medeiros, 2004), and a recent study also reported minor biases for MDA-treated populations of the genes encoding methane-

and ammonia-oxidizing enzymes (*pmoA, amoA* and *mmoX*) in environmental DNA (Erwin et al., 2005). Therefore, our study may not be free of MDA-caused biases, the *dsrAB* gene distribution should be regarded as only qualitative to semi-quantitative.

The amplified bulk DNAs were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and prepared at 70 ng μl^{-1} each, and then used to amplify *dsrAB* sequences (*ca.* 1.9 kb) using ExTaq DNA polymerase (TaKaRa, Otsu, Japan) on a TaKaRa Cycler PERSONAL TP240, with the *dsrAB*-specific primers of DSR1F and DSR4R of Wagner et al. (1998).

The PCR products of the expected sizes were excised and extracted from electrophoresis agarose gels, and cloned using the TOPO TA Cloning Kit containing the cloning vector pCR2.1 and the competent One Shot TOP10 *E. coli* cells (Invitrogen, Carlsbad, San Diego, Calif.). The inserts were amplified by direct PCR analysis from a randomly selected white colony, using M13 primers, and purified with the QIAquick PCR Purification Kit. The Transformants were randomly collected and sequenced bi-directionally by the dideoxy method (Sanger et al., 1977) on a 3730xl DNA Analyzer (Applied Biosystems, Foster, USA) using the sequencing primers 1F1 and 1R1 of Dhillon et al. (2003) as well as the T7promoter and T7R [5'-TTGGTACCGAGCTCGGAT-3'] primers for pCR vectors.

Retrieved sequences were checked for homology to sequences in the DNA Data Bank of Japan (DDBJ; www.ddbj.hig.ac.jp) based on both nucleotide and amino acid BLAST analysis (Altschul et al., 1997). Sequences without homology to target genes were excluded from further analyses. The inferred DsrAB amino acid sequences were aligned using ClustalW (Thompson et al., 1994). Phylogenetic trees were

constructed by the neighbor-joining (Saitou and Nei, 1987), minimum evolution (Rzhetsky and Nei, 1992) and maximum parsimony (Fitch, 1971) algorithm using MEGA 3.1 program (Kumar et al., 2004) and by maximum likelihood (Felenstein, 1995) using PHYLIP 3.6.3. The trees based on DsrAB (*ca.* 600 amino acids; Figure 1) and DsrA (*ca.* 216 amino acids; not shown) sequences were compared to check variable topologies that may be due to chimeras.

The *dsrAB* sequences having >95% amino acid similarities were grouped into operational units of Nankai Trough *dsrAB* (NTd), and the most equidistant sequence within a unit was chosen to represent the unit. The representative sequences were deposited to DDBJ under the accession numbers AB263125 to AB263183.

Results and Discussion

Although sulfate reduction is a dominant anaerobic pathway in subsurface of continental margin (e.g. D'Hondt et al., 2002), only few diversity and distribution of sulfate reducers have been reported (Thomsen et al. 2001; Mauclaire et al., 2004; Schippers and Neretin, 2006). Thus, we have profiled the vertical/horizontal distribution and diversity of the SRP-specific functional gene "*dsrAB*" (Stahl et al. 2002).

A total of 407 clones from 37 PCR-positive (with four negative) sections of five cores (Table 1) were sequenced, 384 of which were identified as *dsrAB* by BLAST homology search. The 382 of *dsrAB* sequences were grouped into 61 operational units (NTd), and 59 units that survived the chimera check were

further grouped into eight phylogenetic clusters, namely Dsr Groups I to VIII (Figures 1 and 2**a**).

The Dsr Group I with 128 clones (33.5% of total) dominated the *dsrAB* population and was affiliated with *dsrAB* of the deltaproteobacterial family *Desulfobacteraceae*. One sub-group (NTd-I14 to -I27; 73 clones) was affiliated with the genera *Desulfobacterium*, *Desulfonema*, *Desulfococcus* and *Desulfosaricina* capable of complete oxidation of organic matter including acetate (Widdel and Bak, 1992) (Figure 1). The other sub-group (NTd-I01 to -I13; 55 clones) formed a separate branch with a 53% bootstrap value from other DsrAB of the family *Desulfobacteraceae* (Figure 1).

The Dsr Group II (55 clones) was affiliated with the deltaproteobacterial family *Syntrophobacteraceae*, and 54 clones were closely related, with amino acid similarities of 85-94%, to the hydrocarbon-rich hydrothermal Guaymas Basin clone B01P021 (AY197431) that forms a monophyletic lineage branched from *Thermodesulforhabdus norvegica* and *Desulforhabdus amnigena* (Dhillon et al., 2003).

The Dsr Group VII (3 clones) was also affiliated with the deltaproteobacterial family, *Desulfobulbuceae*, and the three clones were related to the *dsrAB* clones from the Okinawa Trough hydrothermal vents (AB124919 and AB124920) (Nakagawa et al., 2004). The Dsr Groups I, II and VII occupied 48.7% (186 clones) of total, and it is likely that deltaproteobacterial species dominate the Nankai Trough SRP flora.

The Gram-positive *Firmicutes*-related lineages were found in the Dsr Groups III and IV, 115 and 40 clones, respectively. The Group III formed a branch within a monophyletic cluster consisting of the non-sulfate-reducing Gram-positive firmicute *Pelotomaculum* sp. MGP (Imachi et al., 2006) and the thermophilic sulfate-reducer *Thermodusulfobium narugense* (Mori et al., 2003) (Figure 1). No known

dsrAB of cultured species or environmental clones were related to 114 clones of Group III, with one clone (NTd-III09) showing a 74% amino acid similarity with an anaerobic sludge clone (AY929600). Group IV was closely related to *dsrAB* of *Desulfobacterium anilini*, 39 clones of which were related to a salt marsh clone (AY741568) (Bahr et al., 2005) with 80-89% amino acid similarities, with one clone branched with a freshwater mudflat clone (AY953411) (Leloup et al., 2006). It should be noted that *dsr* is often exposed to lateral gene transfer (Klein et al., 2001), and *dsr* of *D. aniline* and gram-positive SRP may share the same donor linage (Zverlov et al., 2005). The Group IV *dsr* may be a possible donor for them or share the same *dsr* donor lineage with them. However, the possibility should be tested by: isolation of novel deltaproteobacterial SRP that bear Group IV *dsr*; and, comparison of phylogenetic trees based on DsrAB and 16S rRNA genes, both of which have not been done.

The Dsr Groups V, VI and VIII (31, 8 and 2 clones, respectively) were unaffiliated with any cultured SRP but with environmental clones. Group V formed two deeply-branching lineages: one (24 clones) highly affiliated with the Guaymas Basin clones (AY197437, AY197449, and AY197451) (Dhillon et al., 2003) with 81-94% amino acid homologies, and the other (7 clones) related to the metagenomic library clones from the Wadden Sea sediment (CT025835 and CT025836) (Mussmann et al., 2005). The *dsrAB* clones related to Group V are widely present in marine environments, e.g., the Danish coastal sediments (Thomthen et al., 2001), salt marsh (Bahr et al., 2005) and mudflat (Leloup et al., 2006). Group VI was unrelated to any known cultured species/strains and formed a deeply-branching lineage that consists specifically of the Nankai Trough clones with a bootstrap value of 59%. Group VIII was related to an

environmental clone (DQ112192) from the Hong Kong Victoria Harbour sediment.

Four *dsrAB* groups (III, IV, V and VIII; 41% of total clones), which were obtained from the Nankai Trough, were phylogenetically divergent relative to genes from any cultured SRPs (Figure. 1).

Horizontal and vertical distributions of *dsrAB* sequences in five sediment cores (Table 1) were profiled (Figure 2). The deltaproteobacterial Group I was most commonly found in the sampled sediments (Figure 2**a**), detected in all the five cores (Figure 2**b**) and in all the vertical, particularly shallower, sections of three cores (Figure 2**c**). Similarly the deltaproteobacterial Group II clones were abundant in shallower sections. In contrast, the novel Dsr Group III clones tended to increase in number with depth (Figure 2**c**). The Aarhus Bay sediment, Denmark, showed the vertical profile of *dsrAB* clones similar to those of the Nankai Trough sediments (BO03-PC01, BO03-PC02, BO03-PC05 and BO04-PC03); while deltaproteobacterial *dsrAB* clones, particularly of *Desulfobacteraceae*, occur in a shallow zone (21.5 cm below-seafloor), novel *dsrAB* sequences occur in deeper zones (81.5 and 156.5 cm below-seafloor) (Thomsen et al., 2001). Contrary, the Aarhus Bay sediment yielded no Group III clones of the Nankai Trough. Other Dsr Groups showed only unclear patterns of vertical distributions. On the other hand, the core BO04-PC08 (Table 1) showed a notable horizontal distribution. It yielded only 17 clones, but six clones belonged to the Nankai Trough-specific Group VI consisting of only eight clones in total.

In this study, we have revealed the presence of *dsrAB* genes (DNA) in the Nankai Trough sediments, however it is still unclear whether they are active or not. In the future studies, we should consider the *in situ* sulfate reduction activity of SRPs in the Nankai Trough by measuring the Dsr messenger RNA (Dsr

mRNA).

This study presented the occurrence of *dsrAB* genes from diverse lineages in deep seafloor, which increases the choices of genetic materials and information for bioremediation via sulfate reduction (e.g. Lovley et al., 1993; Suyama et al., 2001). Finding of *dsrAB* sequences of firmicute lineages may facilitate use of dsr genes by developing shuttle vectors for *Bacillus subtilis* and sulfate-reducing firmicutes, in addition to existing systems for *Escherichia coli* (gammaproteobacteria) and deltaproteobacterial sulfate-reducers (Wall et al., 1993; Rousset et al., 1998). The *dsrAB* genes from novel lineages may also help understanding deep-sea metagenomics.

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References

Abulencia CB, Wyborski DL, Garcia JA, Podar M, Chen W, Chang SH, Chang HW, Watson D, Brodie EL, Hazen TC, Keller M (2006) Environmental whole-genome amplification to access microbial population in contaminated sediments. Apple Environ Microbiol 72, 3291-3301

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25,

3389-3402

Amann R, Ludwig W, Schleifer K (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59, 143-169

Arakawa S, Sato T, Yoshida Y, Usami R, Kato C (2006) Comparison of the microbial diversity in cold-seep sediments from different depth in the Nankai Trough. J Gen Appl Microbiol 52, 47-54

Bahr M, Crump BC, Klepac-Ceraj V, Teske A, Sogin ML, Hobbie JE (2005) Molecular characterization of sulfate-reducing bacteria in a New England salt marsh. Environ Microbiol 7, 1175-1185

Bowman JP, McCuaig RD (2003) Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. Appl Environ Microbiol 69, 2463–2483

Dean FB, Hosono S, Fang L, Wu X, Faruqi AF, Bray-Ward P, Sun Z, Zong Q, Du Y, Du J, Driscoll M, Song W, Kingsmore SF, Egholm M, Lasken RS (2002) Comprehensive human genome amplification using multiple displacement amplification. Proc Natl Acad Sci USA 99, 5261-5266

Devereux R, Mundfrom GW (1994) A phylogenetic tree of 16S rRNA sequences from sulfate-reducing bacteria in a sandy marine sediment. Appl Environ Microbiol 60, 3437-3439

Dhillon A, Teske A, Dillon J, Stahl DA, Sogin ML (2003) Molecular characterization of sulfate-reducing bacteria in the Guaymas Basin. Appl Environ Microbiol 69, 2765–2772

D'Hondt S, Rutherfod S, Spivack AL (2002) Metabolic activity of subsurface life in deep-sea sediments. Science 295, 2067-2070

D'Hondt S, Jørgensen BB, Miller DJ, Batzke A, Blake R, Cragg BA, Cypionka H, Dickens GR, Ferdelman

T, Hinrichs KU, Holm NG, Mitterer R, Spivack A, Wang G, Bekins B, Engelen B, Ford K, Gettemy G, Rutherford SD, Sass H, Skilbeck CG, Aiello IW, Guèrin G, House C, Inagaki F, Meister P, Naehr T, Niitsuma S, Parkes RJ, Schippers A, Smith DC, Teske A, Wiegel J, Padilla CN, Acosta JLS (2004) Distributions of microbial activities in deep subseafloor sediments. Science 306, 2216-2221

Erwin DP, Erickson IK, Delwiche ME, Colwell FS, Strap JL, Crawford RL (2005) Diversity of oxygenase genes from methane- and ammonia-oxidizing bacteria in eastern Snake River plain aquifer. Apple Environ Microbiol 71, 2016-2025.

Felsenstein J (1995) PHYLIP: phylogeny inference package, version 3.57c. Department of Genetics, University of Washington, Seattle

Fitch WM (1971) Towards defining the course of evolution: Minimum change for a specific tree topology. Systematic Zoology 20, 406-416

Fukuba T, Ogawa M, Fujii T, Naganuma T (2003) Phylogenetic diversity of dissimilatory sulfite reductase genes from deep-sea cold seep sediment. Mar Biotechnol 5, 458-468

Gonzalez JM, Portillo MC, Saiz-Jimenez C (2005) Multiple displacement amplification as pre-polymerase chain reaction (pre-PCR) to process difficult to amplify samples and low copy number sequences from natural environments. Environ Microbiol 7, 1024-1028

Hatchikian EC, Zeikus JG (1983) Characterization of a new type of dissimilatory sulfite reductase present in Thermodesulfobacterium commune. J Bacteriol 153, 1211-1220

Hosono S, Faruqi AF, Dean FB, Du Y, Sun Z, Wu X, Du J, Kingsmore SF, Egholm M, Lasken RS (2003)

Unbiased whole-genome amplification directly from clinical samples. Genome Res 13, 954-964

Imachi H, Sekiguchi Y, Kamagata Y, Loy A, Qiu YL, Hugenholtz P, Kimura N, Wagner M, Ohashi A, Harada H (2006) Non-Sulfate-Reducing, syntrophic bacteria affiliated with Desulfotomaculum cluster I are widely distributed in methanogenic environments. Appl Environ Microbiol 72, 2080-2091

Jørgensen BB (1977) The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol Oceanogr 22, 814-832

Jørgensen BB (1982) Mineralization of organic matter in the sea-bed—the role of sulphate reduction. Nature 296, 643-645

Klein M, Friedrich MW, Roger AJ, Hugenholtz P, Fishbain S, Abicht H, Blackall LL, Stahl DA, Wagner M (2001) Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes. J Bacteriol 183, 6028-6035

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. Brief Bioinform 5, 150-163

Leloup J, Quillet L, Berthe T, Petit F (2006) Diversity of the dsrAB (dissimilatory sulfite reductase) gene sequences retrieved from two contrasting mudflats of the Seine estuary, France. FEMS Microbiology Ecology 55, 230-238

Li L, Guenzennec J, Nichols P, Henry P, Yanagibayashi M, Kato C (1999) Microbial diversity in Nankai Trough sediments at depth of 3,843 m. J Oceanograph 55, 635-642

Llobet-Brossa E, Rosselló-Mora R, Amann R (1998) Microbial community composition of Wadden Sea

sediments as revealed by fluorescence in situ hybridization. Appl Environ Microbiol 64, 2691-2696

Lovley DR, Widman PK, Woodward JC, Phillips EJ. (1993) Reduction of uranium by cytochrome c3 of *Desulfovibrio vulgaris*. Appl Environ Microbiol 59, 3572-3576

Loy A, Kusel K, Lehner A, Drake HL, Wagner M (2004) Microarray and functional gene analyses of sulfate-reducing prokaryotes in low-sulfate, acidic fens reveal cooccurrence of recognized genera and novel lineages. Appl Environ Microbiol 70, 6998-7009

Luthra R, Medeiros LJ (2004) Isothermal multiple displacement amplification *A highly reliable approach for generation unlimited high molecular weight genomic DNA from clinical speciments*. J Mol Diagn 6, 236-242

Mauclaire L, Zepp K, Meister P, Mckenzie J (2004) Direct in situ detection of cells in deep-sea sediment cores from the Peru Margin (ODP Leg 201, Site 1229). Geobiology 2, 217-223

Miller DN, Bryant JE, Madsen EL, Ghiorse WC (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microbiol 65, 4715-4724

Mori K, Kim H, Kakegawa T, Hanada S (2003) A novel lineage of sulfate-reducing microorganisms: Thermodesulfobiaceae fam. nov., *Thermodesulfobium narugense*, gen. nov., sp. nov., a new thermophilic isolate from a hot spring. Extremophiles 7, 283-290

Molitor M, Dahl C, Molitor I, Schafer U, Speich N, Huber R, Deutzmann R, Truper HG (1998) A dissimilatory sirohaem-sulfite-reductase-type protein from the hyperthermophilic archaeon Pyrobaculum islandicum. Microbiology 144, 529–541

Mußmann M, Ishii K, Rabus R, Amann R (2005) Diversity and vertical distribution of cultured and uncultured Deltaproteobacteria in an intertidal mudflat of the Wadden Sea. Environ Microbiol 7, 405–418

Mussmann M, Richter M, Lombardot T, Meyerdierks A, Kuever J, Kube M, Glöckner FO, Amann R (2005) Clustered genes related to sulfate respiration in uncultured prokaryotes support the theory of their concomitant horizontal transfer. J Bacteriol 187, 7126-7137

Nakagawa T, Nakagawa S, Inagaki F, Takai K, Horikoshi K (2004) Phylogenetic diversity of sulfate-reducing prokaryotes in active deep-sea hydrothermal vent chimney structures. FEMS Microbiol Lett 232, 145-152

Pérez-Jiménez JR, Kerkhof LJ (2005) Phylogeography of sulfate-Reducing bacteria among disturbed sediments, disclosed by analysis of the dissimilatory sulfite reductase genes (dsrAB). Appl Environ Microbiol 71, 1004-1011

Purdy KJ, Nedwell DB, Embley TM (2003) Analysis of the sulfate-reducing bacteria and methanogenic archaeal populations in contrasting Antarctic sediments. Appl Environ Microbiol 69, 3181-3191

Ravenschlag K, Sahm K, Knoblauch C, Jørgensen BB, Amann R (2000) Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine arctic sediments. Apple Environ Microbiol 66, 3592-3602

Rousset M, Casalot L, Rapp-Giles BJ, Dermoun Z, de Philip P, Belaich JP, Wall JD (1998) New shuttle vectors for the introduction of cloned DNA in *Desulfovibrio*. Plasmid 39, 114-122.

Rzhetsky A, Nei M (1992) A simple method for estimating and testing minimum-evolution trees. Mol Biol

Evol 9, 945-967

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic tree. Mol Biol Evol 4, 406-425

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74, 5463-5467

Schippers A, Neretin LN (2006) Quantification of microbial communities in near-surface and deeply buried marine sediments on the Peru continental margin using real-time PCR. Environ Microbiol 8, 1251-1260

Stahl DA, Fishbain S, Klein M, Baker BJ, Wagner M (2002) Origins and diversification of sulfate-respiring microorganisms. Antonie Van Leeuwenhoek 81, 189-195

Suyama A, Iwakiri R, Kai K, Tokunaga T, Sera N, Furukawa K. (2001) Isolation and characterization of *Desulfitobacterium* sp. strain Y51 capable of efficient dehalogenation of tetrachloroethene and polychloroethanes. Biosci Biotechnol Biochem 65, 1474-1481

Suzuki MT, Rappé MS, Haimberger ZW, Winfield H, Adair N, Ströbl J, Giovannoni SJ (1997) Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. Appl Environ Microbiol 63, 983-989

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucreic Acid Res 22, 4673-4680

Thomsen TR, Finster K, Ramsing NB (2001) Biogeochemical and molecular signatures of anaerobic

methane oxidation in a marine sediment. Appl Environ Microbiol 67, 1646-1656

Wagner M, Roger AJ, Flax JL, Brusseau GA, Stahl DA (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. J Bacteriol 180, 2975-2982

Wall JD, Rapp-Giles BJ, Rousset M (1993) Characterization of small plasmid from *Desulfovibrio desulfuricans* and its use for shuttle vector construction. J. Bacteriol 175, 4121-4128

Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: The Prokaryotes, 2nd Vol. IV (Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH, Eds.), pp. 3352-3378. Springer-Verlag, New York.

Zverlov V, Klein M, Lucker S, Friedrich MW, Kellermann J (2005) Lateral gene transfer of dissimilatory (Bi)sulfite reductase revisited. J Bacteriol 187, 2203–2208

Table 1. Locations of core collection and sampled core sections.

Core code (water depth) Location	Retrieved core length [cm]	Sampled sections [cm below seafloor] (*dsrAB* sequences detected in **Bold** sections)
BO03-PC01 (2057 m) 33°45.74'N, 136°27.60'E	197	**15-16**, **40-41**, **55-56**, **72-73**, **94-95**, **108-109**, **132-133**, **152-153**, **186-187**, **192-193**
BO03-PC02 (1805 m) 33°50.00'N, 136°25.92'E	140	**20-21**, **37-38**, **50-51**, **75-76**, **90-91**, **120-121**, **130-131**
BO03-PC05 (1186 m) 34°14.06'N, 137°40.25'E	67	**13-14**, **25-26**, **35-36**, **45-46**, **55-56**, **64-65**
BO04-PC03 (2053 m) 33°49.16'N, 136°30.06’E	446.5	**23-28**, **46-48**, **67-69**, **90-92**, **115-117**, **140-142**, **187-189**, **237-239**, **287-289**, **337-339**, **387-389**, **437-439**
BO04-PC08 (1206 m) 34°10.61'N, 137°25.31’E	158	**12-14**, 37-39, **67-69**, 87-89, 112-114, 143-145

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Fig. 1. Phylogenetic tree based on DsrAB amino acids (*ca.* 600 amino acids) from the Nankai Trough deep-sea sediment cores. The tree was constructed using the neighbor-joining method in MEGA 3.1 program. The Dsr amino acid sequences of the genus *Thermodesulfovibrio* (U58122, AF334599) were used as outgroups. Bootstrap values at branching nodes after 1,000 resamplings are shown with more than 50% bootstrap support. The number of clones in each operational unit (NTd) is indicated in parentheses.

Fig. 2. Phylogenetically grouped *dsrAB* clones from the Nankai Trough sediment core samples: **a**) overall composition of total 382 clones; **b**) clone compositions of five cores; and, **c**) clone compositions of depth-sectioned samples. The number of clones in each library is indicated in parentheses.

Fig. 1

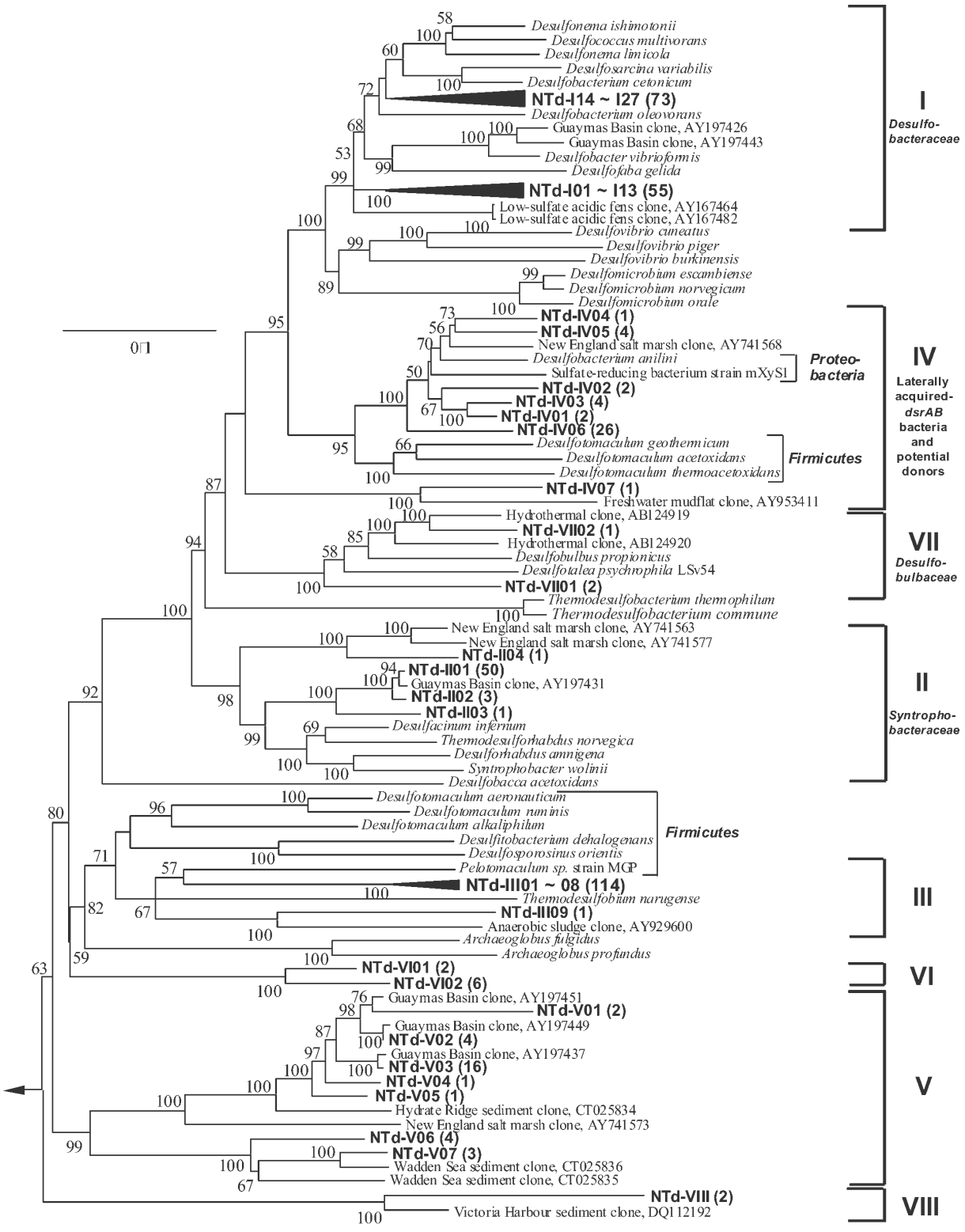
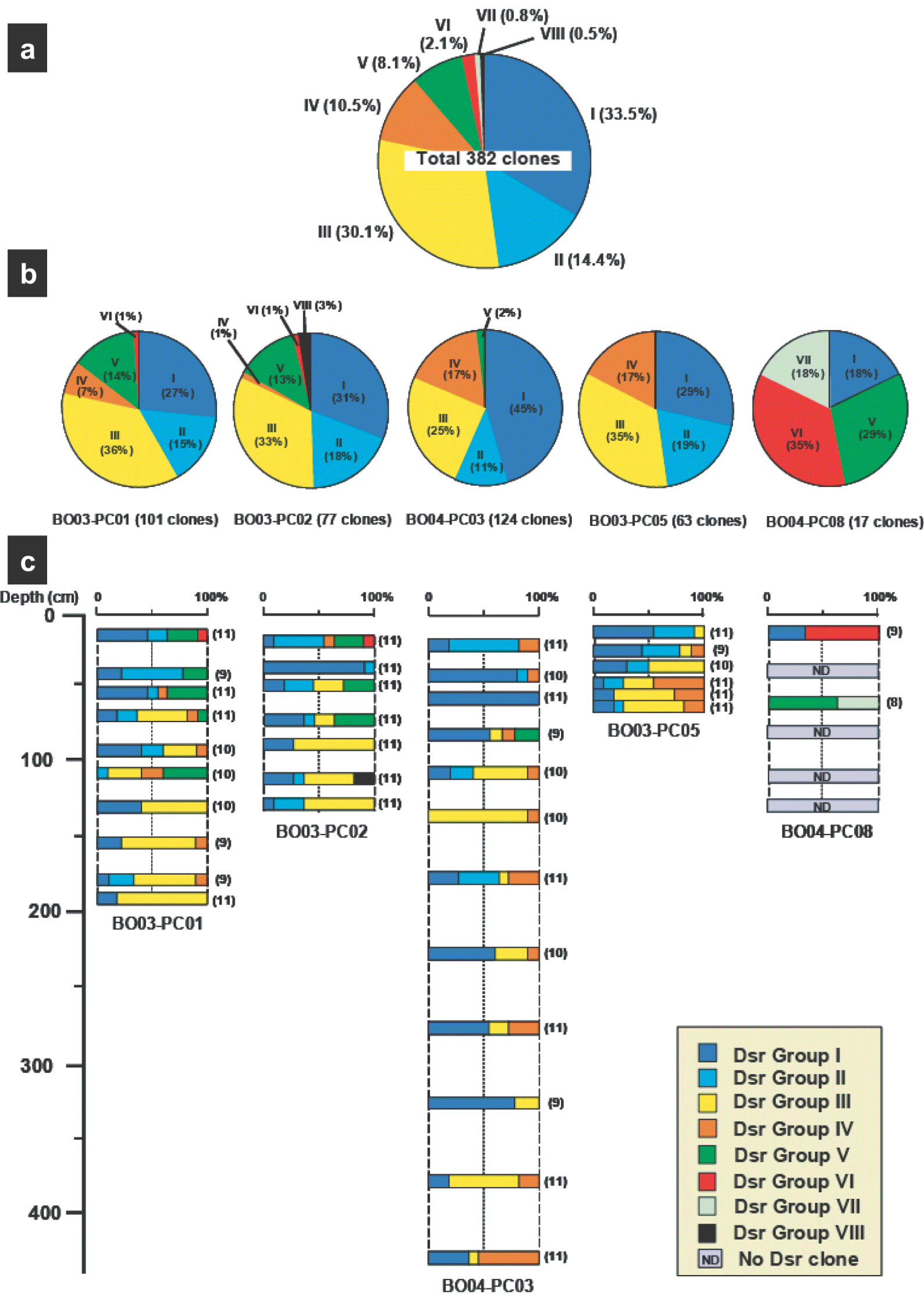

Desulfonema ishimotonii
Desulfococcus multivorans
Desulfonema limicola
Desulfosarcina variabilis
Desulfobacterium cetonicum
NTd-I14 ~ I27 (73)
Desulfobacterium oleovorans
Guaymas Basin clone, AY197426
Guaymas Basin clone, AY197443
Desulfobacter vibrioformis
Desulfofaba gelida
NTd-I01 ~ I13 (55)
Low-sulfate acidic fens clone, AY167464
Low-sulfate acidic fens clone, AY167482
Desulfovibrio cuneatus
Desulfovibrio piger
Desulfovibrio burkinensis
Desulfomicrobium escambiense
Desulfomicrobium norvegicum
Desulfomicrobium orale
0.1
NTd-IV04 (1)
NTd-IV05 (4)
New England salt marsh clone, AY741568
Desulfobacterium anilini
Sulfate-reducing bacterium strain mXyS1
NTd-IV02 (2)
NTd-IV03 (4)
NTd-IV01 (2)
NTd-IV06 (26)
Desulfotomaculum geothermicum
Desulfotomaculum acetoxidans
Desulfotomaculum thermoacetoxidans
NTd-IV07 (1)
Freshwater mudflat clone, AY953411
Hydrothermal clone, AB124919
NTd-VII02 (1)
Hydrothermal clone, AB124920
Desulfobulbus propionicus
Desulfotalea psychrophila LSv54
NTd-VII01 (2)
Thermodesulfobacterium thermophilum
Thermodesulfobacterium commune
New England salt marsh clone, AY741563
New England salt marsh clone, AY741577
NTd-II04 (1)
NTd-II01 (50)
Guaymas Basin clone, AY197431
NTd-II02 (3)
NTd-II03 (1)
Desulfacinum infernum
Thermodesulforhabdus norvegica
Desulforhabdus amnigena
Syntrophobacter wolinii
Desulfobacca acetoxidans
Desulfotomaculum aeronauticum
Desulfotomaculum ruminis
Desulfotomaculum alkaliphilum
Desulfitobacterium dehalogenans
Desulfosporosinus orientis
Pelotomaculum sp. strain MGP
NTd-III01 ~ 08 (114)
Thermodesulfobium narugense
NTd-III09 (1)
Anaerobic sludge clone, AY929600
Archaeoglobus fulgidus
Archaeoglobus profundus
NTd-VI01 (2)
NTd-VI02 (6)
Guaymas Basin clone, AY197451
NTd-V01 (2)
Guaymas Basin clone, AY197449
NTd-V02 (4)
Guaymas Basin clone, AY197437
NTd-V03 (16)
NTd-V04 (1)
NTd-V05 (1)
Hydrate Ridge sediment clone, CT025834
New England salt marsh clone, AY741573
NTd-V06 (4)
NTd-V07 (3)
Wadden Sea sediment clone, CT025836
Wadden Sea sediment clone, CT025835
NTd-VIII (2)
Victoria Harbour sediment clone, DQ112192
I
Desulfo-bacteraceae
IV
Laterally acquired-dsrAB bacteria and potential donors
Proteo-bacteria
Firmicutes
VII
Desulfo-bulbaceae
II
Syntropho-bacteraceae
Firmicutes
III
VI
V
VIII

Fig. 2


a
VII (0.8%)
VI (2.1%)
VIII (0.5%)
V (8.1%)
IV (10.5%)
I (33.5%)
Total 382 clones
III (30.1%)
II (14.4%)
b
VI (1%)
V (14%)
IV (7%)
I (27%)
III (36%)
II (15%)
IV (1%)
VI (1%)
VIII (3%)
V (13%)
I (31%)
III (33%)
II (18%)
V (2%)
IV (17%)
I (45%)
III (25%)
II (11%)
IV (17%)
I (29%)
III (35%)
II (19%)
VII (18%)
I (18%)
VI (35%)
V (29%)
BO03-PC01 (101 clones)
BO03-PC02 (77 clones)
BO04-PC03 (124 clones)
BO03-PC05 (63 clones)
BO04-PC08 (17 clones)
c
Depth (cm)
0
100
200
300
400
0
100%
(11)
(9)
(11)
(11)
(10)
(10)
(10)
(9)
(9)
(11)
BO03-PC01
(11)
(11)
(11)
(11)
(11)
(11)
(11)
BO03-PC02
(11)
(10)
(11)
(9)
(10)
(10)
(11)
(10)
(11)
(9)
(11)
(11)
BO04-PC03
(11)
(9)
(10)
(11)
(11)
(11)
BO03-PC05
(9)
ND
(8)
ND
ND
ND
BO04-PC08
Dsr Group I
Dsr Group II
Dsr Group III
Dsr Group IV
Dsr Group V
Dsr Group VI
Dsr Group VII
Dsr Group VIII
ND
No Dsr clone