Supplementary information:

Depth-Related Differences in Organic Substrate Utilization by Major Microbial Groups in Intertidal Marine Sediment

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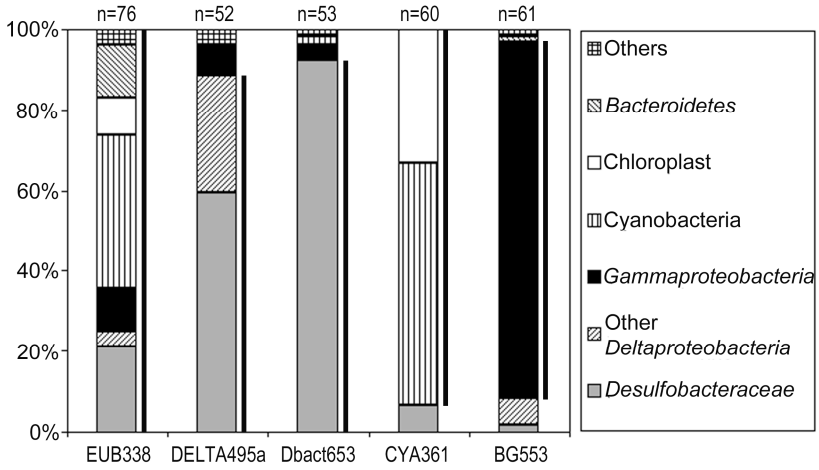

FIG S1 Proportion of clones obtained from unlabeled 16S rRNA (0-2 cm) captured by each probe. Total numbers of clones sequenced are indicated as n. The bar beside each probe column indicates target range of the probe and shows that specificity was better than 90%. Similar results were obtained for the deeper (2-5 cm) layer (1).

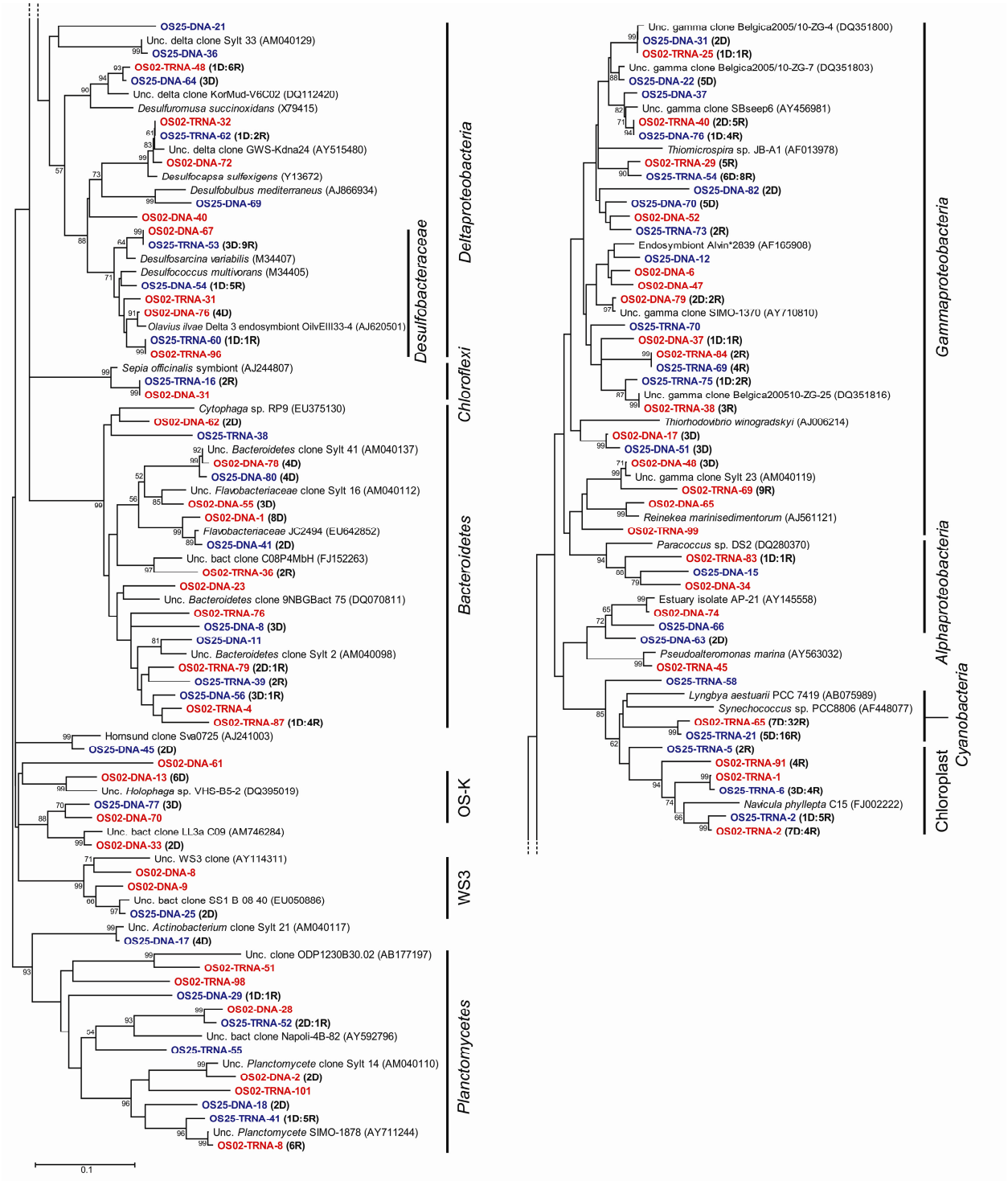
OS25-DNA-21
Unc. delta clone Sylt 33 (AM040129)
OS25-DNA-36
OS02-TRNA-48 (1D:6R)
OS25-DNA-64 (3D)
Unc. delta clone KorMud-V6C02 (DQ112420)
Desulfuromusa succinoxidans (X79415)
OS02-TRNA-32
OS25-TRNA-62 (1D:2R)
Unc. delta clone GWS-Kdna24 (AY515480)
OS02-DNA-72
Desulfocapsa sulfexigens (Y13672)
Desulfobulbus mediterraneus (AJ866934)
OS25-DNA-69
OS02-DNA-40
OS02-DNA-67
OS25-TRNA-53 (3D:9R)
Desulfosarcina variabilis (M34407)
Desulfococcus multivorans (M34405)
OS25-DNA-54 (1D:5R)
OS02-TRNA-31
OS02-DNA-76 (4D)
Olavius ilvae Delta 3 endosymbiont OilvEIII33-4 (AJ620501)
OS25-TRNA-60 (1D:1R)
OS02-TRNA-96
Sepia officinalis symbiont (AJ244807)
OS25-TRNA-16 (2R)
OS02-DNA-31
Cytophaga sp. RP9 (EU375130)
OS02-DNA-62 (2D)
OS25-TRNA-38
Unc. Bacteroidetes clone Sylt 41 (AM040137)
OS02-DNA-78 (4D)
OS25-DNA-80 (4D)
Unc. Flavobacteriaceae clone Sylt 16 (AM040112)
OS02-DNA-55 (3D)
OS02-DNA-1 (8D)
Flavobacteriaceae JC2494 (EU642852)
OS25-DNA-41 (2D)
Unc. bact clone C08P4MbH (FJ152263)
OS02-TRNA-36 (2R)
OS02-DNA-23
Unc. Bacteroidetes clone 9NBGBact 75 (DQ070811)
OS02-TRNA-76
OS25-DNA-8 (3D)
OS25-DNA-11
Unc. Bacteroidetes clone Sylt 2 (AM040098)
OS02-TRNA-79 (2D:1R)
OS25-TRNA-39 (2R)
OS25-DNA-56 (3D:1R)
OS02-TRNA-4
OS02-TRNA-87 (1D:4R)
Hornsund clone Sva0725 (AJ241003)
OS25-DNA-45 (2D)
OS02-DNA-61
OS02-DNA-13 (6D)
Unc. Holophaga sp. VHS-B5-2 (DQ395019)
OS25-DNA-77 (3D)
OS02-DNA-70
Unc. bact clone LL3a C09 (AM746284)
OS02-DNA-33 (2D)
Unc. WS3 clone (AY114311)
OS02-DNA-8
OS02-DNA-9
Unc. bact clone SS1 B 08 40 (EU050886)
OS25-DNA-25 (2D)
Unc. Actinobacterium clone Sylt 21 (AM040117)
OS25-DNA-17 (4D)
Unc. clone ODP1230B30.02 (AB177197)
OS02-TRNA-51
OS02-TRNA-98
OS25-DNA-29 (1D:1R)
OS02-DNA-28
OS25-TRNA-52 (2D:1R)
Unc. bact clone Napoli-4B-82 (AY592796)
OS25-TRNA-55
Unc. Planctomycete clone Sylt 14 (AM040110)
OS02-DNA-2 (2D)
OS02-TRNA-101
OS25-DNA-18 (2D)
OS25-TRNA-41 (1D:5R)
Unc. Planctomycete SIMO-1878 (AY711244)
OS02-TRNA-8 (6R)
0.1
Desulfobacteraceae
Deltaproteobacteria
Chloroflexi
Bacteroidetes
OS-K
WS3
Planctomycetes
Unc. gamma clone Belgica2005/10-ZG-4 (DQ351800)
OS25-DNA-31 (2D)
OS02-TRNA-25 (1D:1R)
Unc. gamma clone Belgica2005/10-ZG-7 (DQ351803)
OS25-DNA-22 (5D)
OS25-DNA-37
Unc. gamma clone SBseep6 (AY456981)
OS02-TRNA-40 (2D:5R)
OS25-DNA-76 (1D:4R)
Thiomicrospira sp. JB-A1 (AF013978)
OS02-TRNA-29 (5R)
OS25-TRNA-54 (6D:8R)
OS25-DNA-82 (2D)
OS25-DNA-70 (5D)
OS02-DNA-52
OS25-TRNA-73 (2R)
Endosymbiont Alvin*2839 (AF165908)
OS25-DNA-12
OS02-DNA-6
OS02-DNA-47
OS02-DNA-79 (2D:2R)
Unc. gamma clone SIMO-1370 (AY710810)
OS25-TRNA-70
OS02-DNA-37 (1D:1R)
OS02-TRNA-84 (2R)
OS25-TRNA-69 (4R)
OS25-TRNA-75 (1D:2R)
Unc. gamma clone Belgica200510-ZG-25 (DQ351816)
OS02-TRNA-38 (3R)
Thiorhodovibrio winogradskyi (AJ006214)
OS02-DNA-17 (3D)
OS25-DNA-51 (3D)
OS02-DNA-48 (3D)
Unc. gamma clone Sylt 23 (AM040119)
OS02-TRNA-69 (9R)
OS02-DNA-65
Reinekea marinisedimentorum (AJ561121)
OS02-TRNA-99
Paracoccus sp. DS2 (DQ280370)
OS02-TRNA-83 (1D:1R)
OS25-DNA-15
OS02-DNA-34
Estuary isolate AP-21 (AY145558)
OS02-DNA-74
OS25-DNA-66
OS25-DNA-63 (2D)
Pseudoalteromonas marina (AY563032)
OS02-TRNA-45
OS25-TRNA-58
Lyngbya aestuarii PCC 7419 (AB075989)
Synechococcus sp. PCC8806 (AF448077)
OS02-TRNA-65 (7D:32R)
OS25-TRNA-21 (5D:16R)
OS25-TRNA-5 (2R)
OS02-TRNA-91 (4R)
OS02-TRNA-1
OS25-TRNA-6 (3D:4R)
Navicula phyllepta C15 (FJ002222)
OS25-TRNA-2 (1D:5R)
OS02-TRNA-2 (7D:4R)
Gammaproteobacteria
Alphaproteobacteria
Chloroplast
Cyanobacteria

FIG S2 Neighbor-joining tree showing the affiliation of clones obtained from 16S rRNA gene and reverse-transcribed 16S rRNA to closely related sequences. Clones designated OS02 are derived from the surface layer, and clones designated OS25 are from the deeper layer. Clones with designations containing DNA and TRNA are derived from DNA and total community RNA, respectively. Clones with 97% sequence similarity were considered to represent similar phylotypes. Numbers and letters in parenthesis indicate number of clones in a phylotype and type of sequences (D for DNA-derived, R for RNA-derived). The rRNA clones from the 2-5 cm zone are derived from Miyatake et al. (1) and are included here for comparison with the other libraries. Bootstrap values represent 1000 replicates and only values greater than 50% are reported. The scale bar indicates 10% difference.

Approximately 35 μg of DNA and 15 μg of RNA per 1 g (dry weight) of sediment were extracted from the surface layer, and somewhat lower amounts from the deeper layer (approximately 15 μg g^{-1} of DNA and 10 μg g^{-1} of RNA). Between 80 and 100 clones were obtained for each of the four clone libraries. Diversity, in terms of the number of phylotypes, appeared to be somewhat lower in the rRNA libraries than the rRNA gene libraries, with 35 and 37 rRNA gene phylotypes, and 27 and 23 rRNA phylotypes for the surface and the deeper layer, respectively (at the 97% sequences similarity level). The rRNA libraries of the two depth layers were significantly different from each other (corrected P value ≤ 0.01, determined by UniFrac significance analysis (2)), but the two rRNA gene libraries were only marginally significantly different (corrected P value = 0.03). Comparing the rRNA and rRNA gene libraries from the same layer also gave significant differences (corrected P value ≤ 0.01).

References:

1. **Miyatake T, MacGregor BJ, Boschker HTS.** 2009. Linking Microbial Community Function to Phylogeny of Sulfate-Reducing Deltaproteobacteria in Marine Sediments by Combining Stable Isotope Probing with Magnetic-Bead Capture Hybridization of 16S rRNA. Appl. Environ. Microbiol. **75:**4927-4935.
2. **Lozupone C, Hamady M, Knight R.** 2006. UniFrac - An online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics **7**. doi:10.1186/1471-2105-7-371.