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High Level of Bacterial Diversity and Novel Taxa in Continental Shelf Sediment

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The bacterial diversity of the continental shelf sediment in the Yellow Sea was investigated by the cloning and sequencing of PCR-amplified 16S rRNA genes. The majority of the cloned sequences were distinct phylotypes that were novel at the species level. The richness estimator indicated that the sediment sample might harbor up to 32 phylum-level taxa. A large number of low-abundance, phylum-level taxa accounted for most of the observed phylogenetic diversity at our study site, suggesting that these low-abundance taxa might play crucial roles in the shelf sediment ecosystem.

Keywords: Uncultured bacteria, diversity, shelf sediment

Marine microorganisms are a major component of global biogeochemical cycles [1, 19], and recent technological developments in marine geochemistry and microbiology have revealed that prokaryotes in the marine sediments play critical roles in the marine biogeochemical cycling [10]. Approximately 5 to 10 billion tons of particulate organic matter sinks into the oceans each year, and most of this is deposited on the continental shelves, which constitute only 8.6% of the total ocean floor [18]. The continental shelves are important sites for the biogeochemical cycles in the ocean because only a small percentage of organic matter is permanently buried, and most of the organic matter is re-mineralized in the shelf sediment. However, to our knowledge, only a limited number of studies have been conducted to investigate the microbial community structure in the continental shelf sediments [3, 4, 15, 17, 27], and the shelf sediments remain undersampled.

The continental shelf in the Yellow Sea was submerged in the course of the post-glacial sea-level rise, and it extends from the Bohai Sea in the north to the East China Sea in the south. The seafloor of the Yellow Sea is relatively smooth at water depths of 80 to 100 m, and the seafloor sediments form a distinct layer of relict sands. Most of the finer sediments in the top layer of the sea floor are winnowed out by tidal currents and waves [16]. In this study, we investigated the bacterial community composition using the cloning and sequencing of 16S rRNA genes amplified from the community DNA extracted directly from the Yellow Sea continental shelf sediment. This paper describes an unexpectedly high level of bacterial diversity and novel taxa observed from the Yellow Sea continental shelf sediment.

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Sampling Site and Phylogenetic Analysis

A sediment core sample was collected from the southeastern Yellow Sea (125°30'E and 33°30'N), west of Je-Ju Island using a gravity corer. Community DNA was directly extracted from the sediment sample using a Power Soil DNA Isolation Kit (MoBio, Solana Beach, CA, USA). The bacterial 16S rRNA genes were PCR-amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-AAGGAGGTGATCCAGCCGCA-3') [23], purified using a QIAquick PCR Purification Kit (Qiagen, Valentia, CA, USA) and cloned using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA). Inserts of randomly selected clones were sequenced multiple times on each strand using an automated capillary DNA sequencer (Applied Biosystems, Foster City, CA, USA). The cloned sequences were checked for possible chimeric origins using Mallard software based on the Pintail algorithm [2]. A few potentially suspicious sequences were excluded in subsequent analyses. The phylum-level phylogenetic positions of the cloned sequences were determined using the naïve Bayesian classifier [34]. RDP's Sequence Match (RDP database) and NCBI-BLAST (GenBank database) were used to find closely related sequences. The phylogenetic similarity

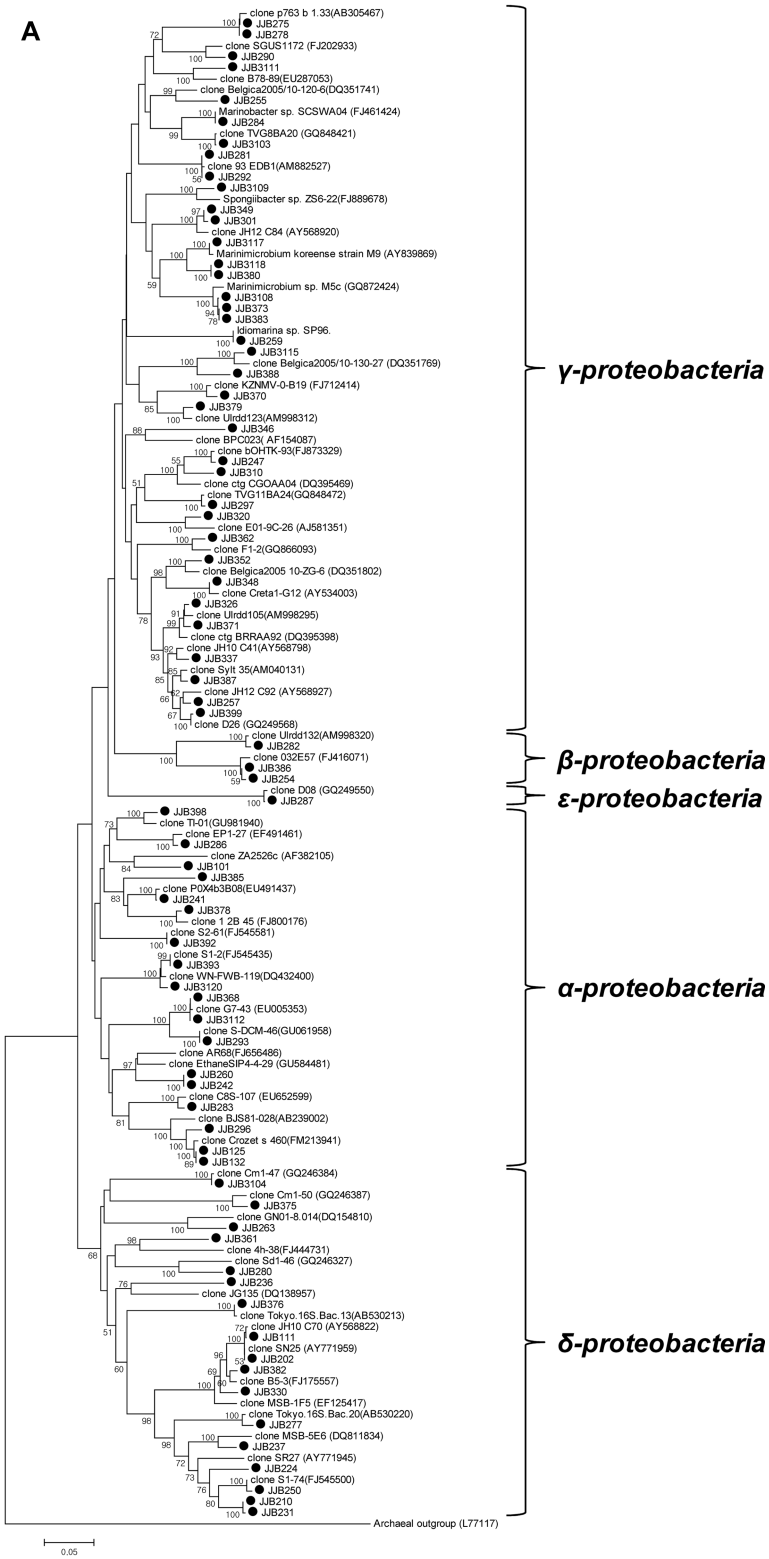

Fig. 1. Phylogenetic positions of the proteobacterial (**A**) and non-proteobacterial (**B**) 16S rRNA clones from the Yellow Sea continental shelf sediment (marked with closed circles; GenBank Accession No. GQ143752–GQ143803 and JN695992–JN696090).
The cloned sequences obtained in this study (marked with closed circles) were compared with the most closely related sequences obtained from the GenBank database. The phylogenetic distances of each sequence were calculated using the Kimura two-parameter model and the tree was constructed using the neighbor-joining algorithm. The numbers at the nodes indicate the bootstrap score (as a percentage) and are shown for frequencies at or above the threshold of 50%. The scale bar represents the expected number of changes per nucleotide position.

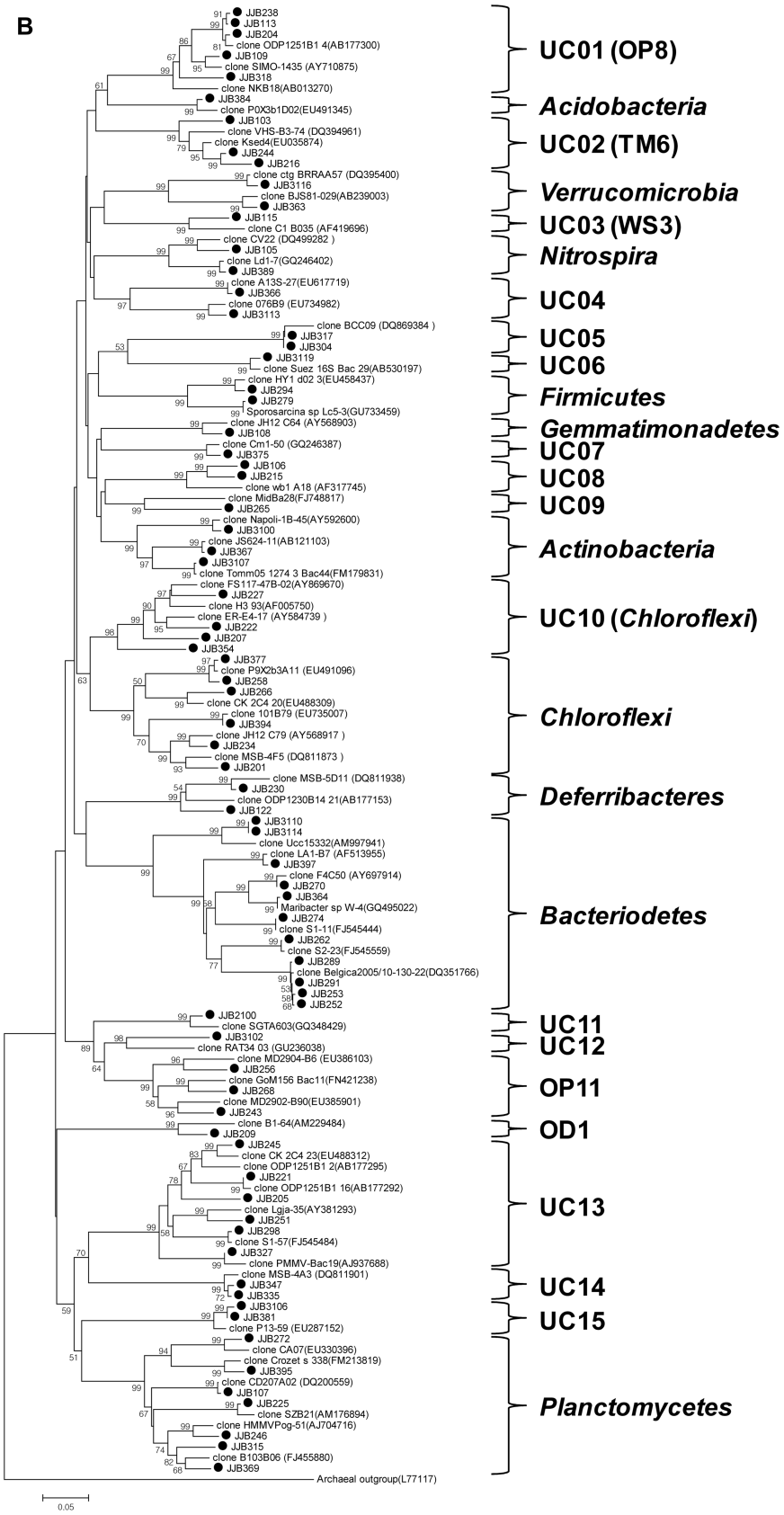

Fig. 1. Continued.

between the clones and the closest relatives were determined using the Kimura two-parameter model. The cloned sequences and reference sequences were edited and aligned using CLUSTALW [31] and then were subjected to phylogenetic reconstruction using MEGA software [21]. The evolutionary distances were calculated using Kimura's two-parameter model [20]. Phylogenetic trees were inferred using the neighbor-joining algorithm and the tree topology was statistically evaluated by 1,000 bootstrap resamplings.

To estimate the richness and coverage at the phylum level, the monophyletic groups (bootstrap score >80%) showing phylum-level divergences in Fig. 1 were defined as operational taxonomic units (OTUs). OTU richness and coverage estimators were calculated using EstimateS software [7]. The OTU richness (S_{Chao1}) was estimated using the nonparametric model of Chao [5] with 100 random sample repetitions; that is, $S_{Chao1} = S_{obs} + (a^2/2b)$, where S_{obs} is the number of OTUs observed, a is the number of singletons, and b is the number of doubletons.

Overview of the Clone Library

A total of 156 clones were obtained from the sediment sample. After removing seven suspicious sequence anomalies detected using the pintail algorithm [2], the remaining 149 clones were subjected to phylogenetic analysis. The closest relatives of the 138 (92.6%) cloned sequences were environmental clones that were primarily (80.6%) recovered from the marine sediment samples (Table 1). The average sequence similarity (calculated using the Kimura two-parameter model) [20] between the clones and their closest relatives was 96.4 ± 3.9%. Sixty-one (40.9%) cloned sequences showed <97% similarity to their closest relatives and 39 (26.2%) of these sequences showed <95% similarity. Phylogenetic reconstruction revealed 28 bootstrap-supported (bootstrap score >80%) major branches in the clone library (Fig. 1), and the depth of the major branches corresponded to the phylum-level divergence (<75% to 80% similarity) suggested by Hugenholtz *et al*. [13, 14].

Phylum-Level Phylogenetic Positions of Cloned Sequences

We initially determined the phylogenetic positions of the cloned 16S rRNA gene sequences using the RDP Bayesian classifier [34]. The phylum-level phylogenetic affiliations of the 112 cloned sequences were assigned using the Bayesian classifier with bootstrap confidence estimates greater than 80% (Table 1). The majority (50.3%) of the assigned clones belonged to the phylum Proteobacteria and the minor groups included phyla Acidobacteria, Actinobacteria, Bacteriodetes, Chloroflexi, Deferribacteres, Firmicutes, Gemmatimonadetes, Nitrospira, Planctomycetes, Verrucomicrobia, and candidate phyla OP11 and OD1. The remaining 37 sequences belonged to unclassified monophyletic groups UC01 through UC15 (Fig. 1B), and the phylogenetic affiliations of these 37 sequences could not be determined using the Bayesian classifier owing to low (<80%) confidence. Although we initially used partial (*ca*. 600 bp) rRNA gene sequences, the Bayesian classifier is known to be accurate down to the genus level and family level for 400 bp and 200 bp partial sequences, respectively [34]. However, the Bayesian classifier tends to provide classification results with low confidence estimates for sequences belonging to regions of the bacterial diversity with less-defined taxonomy. For example, Wang *et al*. [34] reported that in their simulation analysis, the majority of sequences in a library of the phylum Acidobacteria environmental clones could not be classified accurately (<80% confidence estimate), even at the phylum level.

In order to assign the phylum-level phylogenetic affiliations to the clones belonging to the groups UC01 through UC15, near full-length (*ca*. 1,500 bp) sequences of these clones were compared with 41 representative sequences of recognized phyla. The clones of four groups (UC01–03 and UC10) showed sequence similarities greater than 75% (see below for cutoff value) to the recognized phyla (candidate phyla OP8, TM6, WS3, and Chloroflexi). These results were consistent with the groups' phylogenetic positions, as observed in the phylogenetic tree based on the partial sequences. However, the groups UC04–09 and UC11–15 showed <75% similarity to any of the recognized phyla.

High Diversity and Novel Taxa in the Sampling Site

We observed unexpectedly high bacterial diversity in our sediment sample. In the rarefaction analysis where the phylum-level monophyletic clades were defined as operational taxonomic units (OTUs) (Fig. 2), the Chao's richness estimator [5] indicated that our sediment sample

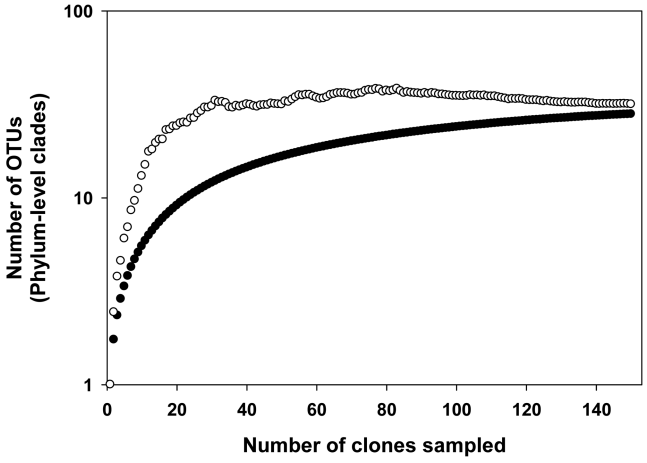

Fig. 2. Phylum-level rarefaction curves for the clone library constructed from the Yellow Sea continental shelf sediment.
The monophyletic clades showing phylum-level divergences were defined as operational taxonomic units (OTUs). The closed circles and open circles indicate the number of OTUs observed (S_{obs}) and the OTU richness (S_{Chao1}) estimated, respectively.

Table 1. The closest relatives and phylogenetic affiliations of PCR-amplified 16S rRNA gene sequences from the Yellow Sea continental shelf sediment.

Clone	Closest relative in GenBank database				Phylogenetic affiliation[b]	
	Sequence definition	Accession no.	Similarity (%)[a]	Isolation environment	Phylum	Confidence estimate (%)
JJB103	Clone VHS-B3-74	DQ394961	91.1	Polluted harbor sediment	UC[c]	
JJB105	Clone CV22	DQ499282	94.8	Cave wall biofilm	Nitrospira	100
JJB106	Clone wb1 A18	AF317745	90.2	Nullarbor caves	UC	
JJB107	Clone CD207A02	DQ200559	99.6	Shallow coastal marine	Planctomycetes	100
JJB108	Clone JH12C64	AY568903	95.2	Intertidal flat	Gemmatimonadetes	97
JJB109	Clone SIMO-1435	AY710875	97.1	Salt marsh	UC	
JJB113	Clone ODP1251B1 4	AB177300	98.6	Methane-hydrate-bearing sediment	UC	
JJB115	Clone C1B035	AF419696	88.8	Hydrothermal basin sediment	UC	
JJB122	Clone ODP1230B14 21	AB177153	91.6	Methane-hydrate-bearing sediment	Deferribacteres	98
JJB201	Clone MSB-4F5	DQ811873	94.0	Mangrove soil	UC	
JJB204	Clone ODP1251B1 4	AB177300	98.9	Methane-hydrate-bearing sediment	UC	
JJB205	Clone ODP1251B1 2	AB177295	90.6	Methane-hydrate-bearing sediment	UC	
JJB207	Clone H3 93	AF005750	89.5	Deep subsurface	UC	
JJB209	Clone B1-64	AM229484	92.1	Oil-polluted microbial mat	OD1	100
JJB2100	Clone SGTA603	GQ348429	95.6	Inlet sediment	UC	
JJB215	Clone wb1A18	AF317745	91.3	Nullarbor caves	UC	
JJB216	Clone VHS-B3-74	DQ394961	92.7	Harbor sediment	UC	
JJB221	Clone ODP1251B1.16	AB177292	99.3	Methane-hydrate-bearing sediment	UC	
JJB222	Clone ER-E4-17	AY584739	93.2	PCBs accumulated sediment	UC	
JJB225	Clone SZB21	AM176894	97.9	Mangrove soil	Planctomycetes	100
JJB227	Clone _ FS117-47B-02	AY869670	93.7	Ridge flank crustal fluids	UC	
JJB230	Clone MSB-5D11	DQ811938	95.4	Mangrove soil	Deferribacteres	100
JJB234	Clone JH12C79	AY568917	95.6	Intertidal flat	UC	
JJB238	Clone ODP1251B1 4	AB177300	98.0	Methane-hydrate-bearing sediment	UC	
JJB243	Clone MD2902-B90	EU385901	93.6	Seafloor sediment	OP11	100
JJB244	Clone Ksed4	EU035874	96.5	Marine sediment	UC	
JJB245	Clone CK_2C4_23	EU488312	96.1	Marine sediment	UC	
JJB246	Clone HMMVPog-51	AJ704716	95.1	Marine sediment	Planctomycetes	100
JJB251	Clone Lgja-35	AY381293	92.5	Landfill soil	UC	
JJB252	Clone Belgica2005/10-130-22	DQ351766	99.5	Marine sediment	Bacteroidetes	100
JJB253	Clone Belgica2005/10-130-22	DQ351766	99.5	Marine sediment	Bacteroidetes	100
JJB256	Clone MD2904-B6	EU386103	89.9	Marine sediment	OP11	99
JJB258	Clone P9X2b3A11	EU491096	97.9	Marine sediment	Chloroflexi	98
JJB262	Clone S2-23	FJ545559	99.6	Marine sediment	Bacteroidetes	100
JJB265	Clone MidBa28	FJ748817	83.8	Estuary sediment	UC	
JJB266	Clone CK_2C4_20	EU488309	94.2	Estuary sediment	Chloroflexi	99
JJB268	Clone GoM156_Bac11	FN421238	92.2	Marine sediment	OP11	93
JJB270	Clone F4C50	AY697914	99.3	Seawater	Bacteroidetes	100
JJB272	Clone CA07	EU330396	97.2	Forest mud	Planctomycetes	100
JJB274	Clone S1-11	FJ545444	100.0	Marine sediment	Bacteroidetes	100
JJB279	*Sporosarcina* sp. Lc5-3	GU733459	99.8	Isolate	Firmicutes	100
JJB289	Clone Belgica2005/10-130-22	DQ351766	99.5	Marine sediment	Bacteroidetes	100
JJB291	Clone Belgica2005/10-130-22	DQ351766	99.6	Marine sediment	Bacteroidetes	100
JJB294	Clone HY1_d02_3	EU458437	97.9	Hyena feces	Firmicutes	100
JJB298	Clone S1-57	FJ545484	99.3	Marine sediment	UC	
JJB304	Clone BCC09	DQ869384	97.1	Marine sediment	UC	
JJB315	Clone CD207A02	DQ200559	87.8	Marine sediment	Planctomycetes	100
JJB317	Clone BCC09	DQ869384	97.1	Marine sediment	UC	
JJB318	Clone NKB18	AB013270	91.0	Marinesediments	UC	

Table 1. Continued.

Clone	Closest relative in GenBank database				Phylogenetic affiliation[b]	
	Sequence definition	Accession no.	Similarity (%)[a]	Isolation environment	Phylum	Confidence estimate (%)
JJB327	Clone PMMV-Bac19	AJ937688	98.1	Hydrocarbon-discharging mud volcano	UC	
JJB335	Clone MSB-4A3	DQ811901	98.1	Mangrove soil	UC	
JJB347	Clone MSB-4A3	DQ811901	97.1	Mangrove soil	UC	
JJB363	Clone BJS81-029	AB239003	96.8	Cold seep sediment	Verrucomicrobia	100
JJB364	*Maribacter* sp. W-4	GQ495022	99.8	Isolate	Bacteroidetes	100
JJB366	Clone A13S-27	EU617719	99.6	Marine sediment	UC	
JJB367	Clone JS624-11	AB121103	99.5	Cold seep sediment	Actinobacteria	84
JJB369	Clone B103B06	FJ455880	94.7	Marine sediment	Planctomycetes	100
JJB375	Clone Cm1-50	GQ246387	97.2	Marine sediment	UC	
JJB377	Clone P9X2b3A11	EU491096	99.3	Sea floor lavas	Chloroflexi	99
JJB381	Clone P13-59	EU287152	98.4	Arctic surface sediment	UC	
JJB384	Clone P0X3b1D02	EU491345	98.9	Sea floor lavas	Acidobacteria	100
JJB389	Clone Ld1-7	GQ246402	98.9	Marine sediment	Nitrospira	97
JJB394	Clone 101B79	EU735007	99.4	Marine sediment	UC	
JJB395	Clone Crozet_s_338	FM213819	96.2	Marine sediment	Planctomycetes	100
JJB397	Clone LA1-B7	AF513955	99.1	Lake water	Bacteroidetes	100
JJB3100	Clone Napoli-1B-45	AY592600	98.3	Mud volcano sediment	UC	
JJB3102	Clone RAT34_03	GU236038	75.6	Soil	UC	
JJB3106	Clone P13-59	EU287152	97.8	Arctic surface sediment	UC	
JJB3107	Clone Tomm05_1274_3_Bac44	FM179831	99.6	Marine sediment	Actinobacteria	95
JJB3110	Clone Ucc15332	AM997941	95.6	Basin sediment	Bacteroidetes	100
JJB3113	Clone 076B9	EU734982	96.3	Basin sediment	UC	
JJB3114	Clone Ucc15332	AM997941	95.6	Basin sediment	Bacteroidetes	100
JJB3116	Clone ctg_BRRAA57	DQ395400	98.6	Marine coral	Verrucomicrobia	100
JJB3119	Clone Suez.16S.Bac.29	AB530197	98.6	Marine sediment	UC	
JJB101	Clone ZA2526c	AF382105	89.3	Marine	Proteobacteria	100
JJB111	Clone SN25	AY771959	99.8	Intertidal mud flat	Proteobacteria	100
JJB125	Clone BJS81-028	AB239002	95.1	Cold seep sediment	Proteobacteria	100
JJB132	Clone BJS81-028	AB239002	95.1	Cold seep sediment	Proteobacteria	100
JJB202	Clone SN25	AY771959	100.0	Intertidal mud flat	Proteobacteria	100
JJB210	Clone JH10 C41	AY568798	92.6	Intertidal flat	Proteobacteria	100
JJB224	Clone JH10 C41	AY568798	89.2	Intertidal flat	Proteobacteria	100
JJB231	Clone JH10 C41	AY568798	92.4	Intertidal flat	Proteobacteria	100
JJB236	Clone JG135	DQ138957	86.6	Paddy soil	Proteobacteria	100
JJB237	Clone MSB-5E6	DQ811834	94.8	Mangrove soil	Proteobacteria	99
JJB241	Clone P0X4b3B08	EU491437	99.6	Sea floor lavas	Proteobacteria	100
JJB242	Clone EthaneSIP4-4-29	GU584481	91.9	Hydrocarbon seep sediment	Proteobacteria	100
JJB247	Clone bOHTK-93	FJ873329	99.3	Marine sediment	Proteobacteria	100
JJB250	Clone S1-74	FJ545500	99.5	Marine sediment	Proteobacteria	100
JJB254	Clone 032E57	FJ416071	98.7	Marine sediment	Proteobacteria	100
JJB255	Clone Belgica2005/10-120-6	DQ351741	96.5	Marine sediment	Proteobacteria	100
JJB257	Clone JH12_C92	AY568927	99.1	Marine sediment	Proteobacteria	100
JJB259	*Idiomarina* sp. SP96	FJ404759	100.0	Isolate	Proteobacteria	100
JJB260	Clone AR68	FJ656486	91.4	Marine sediment	Proteobacteria	100
JJB263	Clone GN01-8.014	DQ154810	91.6	Microbial Mat	Proteobacteria	89
JJB275	Clone p763_b_1.33	AB305467	99.4	Marine sediment	Proteobacteria	100
JJB277	Clone Tokyo.16S.Bac.20	AB530220	98.6	Marine sediment	Proteobacteria	100
JJB278	Clone p763_b_1.33	AB305467	99.4	Marine sediment	Proteobacteria	100
JJB280	Clone Sd1-46	GQ246327	91.1	Marine sediment	Proteobacteria	98
JJB281	Clone 93 EDB1	AM882527	99.8	Water treatment plant sediment	Proteobacteria	100

Table 1. Continued.

Clone	Closest relative in GenBank database				Phylogenetic affiliation[b]	
	Sequence definition	Accession no.	Similarity (%)[a]	Isolation environment	Phylum	Confidence estimate (%)
JJB282	Clone Ulrdd132	AM998320	99.3	Basin sediment	Proteobacteria	100
JJB283	Clone C8S-107	EU652599	98.6	Marine sediment	Proteobacteria	100
JJB284	*Marinobacter* sp. SCSWA04	FJ461424	100.0	Isolate	Proteobacteria	100
JJB286	Clone EP1-27	EF491461	98.6	Marine sediment	Proteobacteria	100
JJB287	Clone D08	GQ249550	99.8	Marine sediment	Proteobacteria	100
JJB290	Clone SGUS1172	FJ202933	96.5	Marine coral	Proteobacteria	100
JJB292	Clone 93 EDB1	AM882527	99.8	Water treatment plant sediment	Proteobacteria	100
JJB293	Clone S-DCM-46	GU061958	100.0	Sea water	Proteobacteria	100
JJB296	Clone Crozet_s_460	FM213941	97.9	Marine sediment	Proteobacteria	100
JJB297	Clone TVG11BA24	GQ848472	99.5	Marine sediment	Proteobacteria	100
JJB301	Clone JH12C84	AY568920	98.0	Intertidal flat	Proteobacteria	100
JJB310	Clone ctgCGOAA04	DQ395469	93.8	Not available	Proteobacteria	99
JJB320	Clone E01-9C-26	AJ581351	97.4	Mediterranean sponges	Proteobacteria	90
JJB326	Clone ctgBRRAA92	DQ395398	98.4	Not available	Proteobacteria	100
JJB330	Clone MSB-1F5	EF125417	96.1	Mangrove soil	Proteobacteria	100
JJB337	Clone ctg BRRAA92	DQ395398	96.0	Not available	Proteobacteria	100
JJB346	Clone BPC023	AF154087	90.6	Hydrocarbon seep sediment	Proteobacteria	100
JJB348	Clone Creta1-G12	AY534003	99.3	Eastern Mediterranean Sea sediment	Proteobacteria	100
JJB349	Clone JH12C84	AY568920	98.0	Intertidal flat	Proteobacteria	100
JJB352	Clone Belgica2005 10-ZG-6	DQ351802	97.0	Marine sediments	Proteobacteria	100
JJB361	Clone 4h-38	FJ444731	87.0	Cotton rizosphere	Proteobacteria	97
JJB362	Clone F1-2	GQ866093	96.9	Gut	Proteobacteria	100
JJB368	Clone G7-43	EU005353	99.8	Artificial marine sediment	Proteobacteria	100
JJB370	Clone KZNMV-0-B19	FJ712414	98.6	Mud volcano sediment	Proteobacteria	100
JJB371	Clone Ulrdd105	AM998295	98.4	Basin sediment	Proteobacteria	100
JJB373	*Marinimicrobium* sp. M5c	GQ872424	98.3	Seashore soil	Proteobacteria	100
JJB376	Clone Tokyo.16S.Bac.13	AB530213	99.8	Marine sediment	Proteobacteria	100
JJB378	Clone 12B45	FJ800176	98.4	Marine sediment	Proteobacteria	100
JJB379	Clone Ulrdd123	AM998312	98.2	Basin sediment	Proteobacteria	100
JJB380	*Marinimicrobium koreense* strain M9	AY839869	95.2	Isolate	Proteobacteria	100
JJB382	Clone B5-3	FJ175557	98.6	Hydrogas seep sediment	Proteobacteria	100
JJB383	*Marinimicrobium* sp. M5c	GQ872424	98.3	Seashore soil	Proteobacteria	100
JJB385	Clone P0X3b1D02	EU491345	90.6	Seafloor lavas	Proteobacteria	100
JJB386	Clone 032E57	FJ416071	98.7	Marine sediment	Proteobacteria	100
JJB387	Clone Sylt 35	AM040131	98.7	Sandy sediment	Proteobacteria	100
JJB388	Clone Belgica2005/10-130-27	DQ351769	92.7	Marine sediment	Proteobacteria	100
JJB392	Clone S2-61	FJ545581	100.0	Marine sediment	Proteobacteria	100
JJB393	Clone S1-2	FJ545435	100.0	Marine sediment	Proteobacteria	100
JJB398	Clone TI-01	GU981940	97.5	Mangrove soil	Proteobacteria	100
JJB399	Clone D26	GQ249568	99.8	Marine sediment	Proteobacteria	100
JJB3103	Clone TVG8BA20	GQ848421	99.8	Marine sediment	Proteobacteria	100
JJB3104	Clone Cm1-47	GQ246384	99.8	Marine sediment	Proteobacteria	96
JJB3108	*Marinimicrobium* sp. M5c	GQ872424	98.5	Isolate	Proteobacteria	100
JJB3109	*Spongiibacter* sp. ZS6-22	FJ889678	96.0	Isolate	Proteobacteria	100
JJB3111	Clone B78-89	EU287053	94.5	Arctic surface sediment	Proteobacteria	100
JJB3112	Clone G7-43	EU005353	99.8	Artificial marine sediment	Proteobacteria	100
JJB3115	Clone Belgica2005/10-130-27	DQ351769	97.5	Marine sediment	Proteobacteria	100
JJB3117	*Marinimicrobium koreense* strain M9	AY839869	100.0	Isolate	Proteobacteria	100
JJB3118	*Marinimicrobium koreense* strain M9	AY839869	95.2	Isolate	Proteobacteria	100
JJB3120	Clone WN-FWB-119	DQ432400	98.8	Lake water	Proteobacteria	100

might harbor up to 31.6 phylum-level taxa (S_{Chao1}, 31.6; S_{obs}, 28). Moreover, the majority (40.7%) of the cloned 16S rRNA gene sequences were distinct phylotypes (sequence similarity <97%). In general, sediment and soil environments have high microbial diversity because of their spatial heterogeneity characteristics [32]. The spatial heterogeneity allows resources to be partitioned to form various microenvironments (niches) that increase the complexity of the microbial diversity. However, the level of diversity observed in this study was much higher than that observed in other studies of marine sediment samples [11, 12, 15, 22, 25, 26, 35]. To our knowledge, this high level of bacterial diversity has previously only been observed in marine sediment samples from the Antarctic continental shelf and the Guaymas basin [4, 8, 30] by using conventional molecular methods. A large number of low-abundance phylum-level taxa accounted for most of the observed phylogenetic diversity at our study site, although temporal variations might exist. We observed a "rare biosphere" [9, 28] in our sediment sample in spite of the relatively small size of our clone library, suggesting that these low-abundance taxa might play crucial roles in the shelf sediment ecosystem.

We also found that a significant portion of the bacteria inhabiting the continental shelf sediment in the Yellow Sea might be novel. Approximately half of the bacteria in our sample have not been cultured or described previously, as evident by the relatively low 16S rRNA gene sequence similarities (96.4±3.9%) of the cloned sequences to their closest relatives in the database. Although the classification of bacteria cannot be completed by the 16S rRNA gene sequence comparison alone, the marginal values of the 16S rRNA gene sequence similarity for species-level and genus-level delineations were estimated to be 97–98% and 95%, respectively [13, 24, 33]. Based on these estimates, 61 (40.9%) sequences in our clone library appeared to be novel at the species level, and 39 sequences (26.2%) could be considered novel at the genus level.

The reason why such significant numbers of novel taxa were found in this environment is unclear, but we developed two hypothetical explanations. First, sediment in the Yellow Sea continental shelf might create a unique environment for microorganisms compared with other marine sediments, which would facilitate the evolution of relatively novel taxa. Therefore, many endemic populations might inhabit this marine environment. However, this explanation might be less probable because the bacterial endemicity was observed only at the genotypic level in soil environments [6], and the spatial isolation would be unlikely in the marine sediments owing to the constant aqueous contact [29]. Second, the size of the current sequence database might not be large enough to find the "true" closest relatives of our clones. Since studies on the bacterial diversity of marine sediments have only begun recently, only a limited portion of 16S rRNA gene sequences have been recovered from the marine sediments, and therefore the 16S rRNA gene sequences that are "truly" closely related to our cloned sequences might not have been deposited in the public databases yet.

Concluding Remarks

We observed that the Yellow Sea continental shelf sediment harbored unexpectedly high bacterial diversity, and the majority of the cloned 16S rRNA gene sequences were novel at the species level and genus level. In our clone library, the high diversity consisted of a large number of low-abundance phylum-level taxa. Although the ecological roles of the low-abundance taxa are currently unclear, they represent a source of genetic diversity and may play an important role in the shelf sediment ecosystem. Genomic/metagenomic sequence analyses, as well as efforts to culture and characterize previously uncultured bacteria, will provide additional information on marine microbial diversity and the taxonomic outline of bacteria which will subsequently help us to understand their ecological roles in marine habitats.

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