

Chapter III

Physiological characterization of aerobic culturable bacteria in the
intestine of the sea cucumber *Apostichopus japonicus*

3.1 Introduction

Sea cucumbers are found on various sea environments like sea floors from deep sea to intertidal areas (Foster and Hodgson, 1995; Uthicke et al., 2009). Sea cucumbers belong to the phylum Echinodermata and they mainly feed on detritus containing organic matter, microalgae, and bacteria (Massin, 1982; Moriarty, 1982; Yingst, 1976).

Gut microorganisms were important for digestion of diets, but the relationships between host sea cucumbers and their gut bacteria and bacterial functions were not still clear. Researches on bacteria associated with sea cucumbers were reported only for *Holothuria atra* and *Molpadia musculus* (Amaro et al., 2009; Ward-Rainey et al., 1996). Ward-Rainey et al. reported preliminary research on aerobic bacterial flora of *Holothuria atra*. In their research, only 23 isolates were characterized by 16S rRNA gene sequences analysis (the first 300 nucleotides) and they were affiliated to the genera *Vibrio* and *Bacillus*. It was reported that the bacterial community of an abyssal holothurian, *Molpadia musculus* was analyzed using the non-culturing methods (Amaro et al., 2009). Amaro et al. found that the gut bacterial composition was similar to that of the organic matter-rich sediments and members of the phylum Bacteroidetes dominated in the bacterial community (Amaro et al., 2009). They recently found that a substantial number of bacterial OTUs (Operational Taxonomic Unit) were associated uniquely with the gut contents and suggested the possibility of wide and highly diversified interactions between prokaryotes and deep-sea holothurians (Amaro et al., 2012). Recently Enomoto et al. reported that Gammaproteobacteria members containing *Vibrio* spp. were isolated

as culturable bacteria from the intestine of *Apostichopus japonicus* (Enomoto et al., 2012). Using the molecular techniques, they also found that Proteobacteria members were main metabolically active microbial populations in the intestine of *Apostichopus japonicus*.

In this paper, I have isolated many various aerobic culturable bacteria associated with *Apostichopus japonicus* using different culture conditions and investigated their diversity and physiological characters including the tests for polysaccharide degradation ability of the isolates to understand the digestive symbiosis in sea cucumbers.

3.2 Materials and Methods

Sample collection. Six kinds of *Apostichopus japonicus* samples (black adult, green adult, black small, green small, black juvenile, and green juvenile sea cucumbers), the sea sediment and the seawater were collected at coastal waters of Kushima, Omura, Nagasaki, Japan on January 28, 2011 (Fig.1). The genetic relationship between black and green types in *Apostichopus japonicus* was examined using 11 microsatellite markers and it was concluded that sympatric black and green types belonged to the same population (Kanno et al., 2006). The surface water temperature was 7.4°C and the salinity of surface water was 2.85‰ at the sampling point. Samples were collected at the water depth of 4 m. The temperature and the salinity of the water depth of 4 m were 7.1°C and 2.93‰, respectively. The surface water temperature and 50 m depth water temperature of open sea near Nagasaki area were 13-15°C (These data were obtained

from Japan Meteorological Agency). The samples were immediately transferred and aseptically dissected in our laboratory. Whole intestine was excised from the animal body aseptically using sterilized instruments. The weights of whole intestines of *Apostichopus japonicus* samples (black adult, green adult, black small, green small, black juvenile, and green juvenile sea cucumbers) were 54.0g, 39.4g, 5.5g, 5.5g, 1.4g and 1.7g, respectively. To isolate bacteria from both intestinal wall and contents, the intact guts were crushed and mixed enough and the same weight of 3.5% saline solution was added to the mixtures. The gut suspensions thus obtained were used for isolation of bacteria. The same weight of 3.5% saline solution was added to the sediment sample and the mixture was used for isolation of bacteria. The seawater sample was used directly for isolation of bacteria. Fifty μ l of samples was spread on each plate and the plates were aerobically incubated at 30°C for two weeks.

Growth media. Luria-Bertani medium (LB) and Horikoshi medium were used with slight modifications. Polysaccharides such as carboxymethyl cellulose sodium salt (CMC) (Wako pure chemicals, Osaka, Japan), xylan (Sigma), sodium alginate (Wako pure chemicals, Osaka, Japan) and soluble starch (nacalai tesque, Kyoto, Japan) were added to Horikoshi medium as carbon sources (final concentration 1%).

LB solid medium (pH 7) contained 1% tryptone (Difco), 0.5% yeast extract (Difco), 3.5% NaCl, and 1.5 % agar (Wako pure chemicals, Osaka, Japan). Horikoshi solid medium (pH 7) contained 1% polysaccharide, 0.5% peptone (BD), 0.5% yeast extract, 0.1% KH_2PO_4 , 0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3.5% NaCl, and 2% agar. Sodium alginate

solid medium contained 2.5% agar. For 10% NaCl media, NaCl concentration of growth media was 10% instead of 3.5%.

For alkaline agar plates, Na₂CO₃ (autoclaved separately) was added to neutral agar medium (final pH: pH10.3-10.5). Na₂CO₃ concentration of alkaline plate was 1%.

Isolation of bacteria. In order to isolate various bacteria, the gut suspension was directly plated on agar plates without enrichment culture. High salt concentration or high pH were used for isolation conditions to isolate various bacteria because marine water is semi-alkaline pH and contains 3.5% NaCl. Seventeen different media were prepared by combination of pH, NaCl concentration and carbon source (Supplementary Table 1). The plates were incubated at 30 °C aerobically for two weeks to obtain slowly growing bacteria. Bacteria were isolated from each plate, purified and stored in slants for further analysis.

Physiological and biochemical characteristics of isolates.

Polysaccharide degradation activities were detected by plate methods using starch (S), CMC, alginate (AL), xylan (XL) or agar as substrate. The following plates were prepared for detection of enzyme activities.

1. Neutral agar plates

1-1. Amylase detection: Horikoshi agar medium containing 1% potato starch instead of soluble starch was used for amylase detection. Amylase-producing colony showed turbid halo around a colony.

1-2. Cellulase detection: Basic neutral agar medium for cellulase detection contained 0.1% CMC, 3.7% marine broth, 0.6% MgCl₂ · 6H₂O, 1.5% agar, 1.6% NaCl, 0.0015%

congo-red, adjust pH to 7.0 with 1N NaOH. Clear zone around a colony suggested cellulase activity.

1-3. Alginate lyase detection: The basic neutral agar medium for alginate lyase detection, contained 1% sodium alginate, 3% NaCl, 0.07% KCl, 0.26% MgSO₄, 0.5% MgCl₂, 0.1% CaSO₄, 0.5% peptone, 0.01% ferric phosphate, 0.1% yeast extract, 2% agar; adjust the pH to 7.0 with 1N NaOH. After two weeks' incubation at 30°C, 70% ethanol was filled into plates. A clear zone around the colony indicated the presence of alginatelyase.

1-4. Xylanase detection: Horikoshi agar medium containing 1% xylan was used for xylanase detection. Xylanase-producing colony showed clear zone around a colony.

1-5. Agarase detection: Horikoshi agar medium without polysaccharide was used for agarase detection. Agarase-producing colony showed dent around a colony.

2. Alkaline agar plates

For alkaline agar plates, Na₂CO₃ (autoclaved separately) was added to neutral agar medium (final pH: pH10.3-10.5). Na₂CO₃ concentration of alkaline plate was 1%.

All isolates were tested for salt tolerance: 0%, 3.5%, 10%, 15%, 20%, 25% NaCl (w/v), pH tolerance (pH7 and pH10) and effect of oxygen. Growth ability at various conditions of salinity or pH was measured at 30°C for two weeks. The isolates were divided into two groups by effect of pH on growth, neutrophilic bacteria (NE) that grew only at pH7, and alkaliphilic bacteria (ALK) that grew both at pH 7 and pH10.

Anaerobic growth was examined using gaspak (COSMO BIO) at 30°C for two weeks, and then growth condition was changed to the aerobic condition at 30°C for two weeks. The isolates were assigned to three groups, facultative anaerobic bacteria (FA),

anaerobic tolerant bacteria (AT) and aerobic bacteria (A). Facultative anaerobic bacteria form colony in both aerobic and anaerobic cultivation. Anaerobic tolerant bacteria do not form colony in anaerobic condition for two weeks but form colony in aerobic cultivation after the anaerobic cultivation. Aerobic bacteria do not form colony in anaerobic condition for two weeks and also do not form colony in aerobic cultivation after the anaerobic cultivation.

Molecular identification of the isolates.

Partial analysis of 16S ribosomal RNA (rRNA) gene of the isolates was carried out. The 16S rRNA gene was amplified using bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTTGTTACGACTT-3') and the purified PCR product was sequenced with dideoxynucleotide chain-termination method using 3130 or 3730 DNA sequencer (Applied Biosystems). Primers 27F, 520R (5'-ACCGCGGCTGCTGGC-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') were used in gene sequencing reactions. Sequences of the partial 16S rRNA genes were assembled and edited using Sequencher (version 4.10.1 demo, Gene Codes Corporation) and MacVector (version 10.0.2). Nucleotide sequences of the partial 16S rRNA genes have been submitted to GenBank/EMBL/DDBJ databases under accession numbers AB734817 through AB735047 (Supplementary Table 2; see Web site).

The partial 16S rRNA gene sequences were compared with other sequences in DDBJ database using BLAST program and compared with type strain sequences in Ribosomal database project (RDP). When an isolate showed $\geq 97\%$ identity with a

certain type strain, the isolate was assigned to the species. When an isolate showed <97% identity with any type strain sequences, the isolate was assigned to the tentative species. Pairwise similarity values were calculated by using Sørensen similarity index: $S=2ab/(a+b)$, where a and b are the number of species in any two categories and ab is the number of common species (Wolda, 1981). A similarity value of 1 indicates that species compositions are identical and a similarity value of 0 indicates that no species are shared.

3.3 Result

Isolation of bacteria

In order to isolate various bacteria, the gut suspensions from samples were directly plated on agar plates without enrichment culture and 17 isolation media were used (Supplementary Table 1; see Web site). Number of colony forming units (cfu)/ g of gut suspensions in sea cucumbers was 1.3×10^4 cfu/g to 2.7×10^4 cfu/g in Horikoshi medium (pH7). Number of cfu/g of the sea sediment was 5.5×10^4 cfu/g in Horikoshi medium (pH7) but number of cfu/g of the seawater was 8.6×10^4 cfu/g in Horikoshi medium (pH7). The viable counts of the sea sediment were similar to those of the gut suspensions, but very low viable counts in the seawater sample.

Total 1133 isolates were purified and analyzed regarding to physiological characteristics. Among them, 231 isolates were analyzed phylogenetically using partial 16S rRNA gene sequences. (Supplementary Table 2; see Web site)

Phylogenetic analysis of bacterial isolates

The partial 16S rRNA gene sequences were compared with type strain sequences in database (Table 1, Supplementary Table 2; see Web site). Based on analysis of partial 16S rRNA gene sequences, 231 isolates from various samples were classified into 53 species with the criterion of 97% sequence identity with type strain species. The 53 species affiliated to the phyla Firmicutes (42 species), Proteobacteria (9 species), and Actinobacteria (2 species). Twelve genera of the phylum Firmicutes belonged to the families Bacillaceae 1 (*Bacillus*), Bacillaceae 2 (*Oceanobacillus*, *Virgibacillus*, *Gracilibacillus* and *Halobacillus*) and Planococcaceae (*Lysinibacillus*, *Planococcus* and *Sporosarcina*). The species of the genus *Bacillus* were mainly *Bacillus aryabhatai*, *Bacillus clausii*, *Bacillus hunanensis*, *Bacillus licheniformis*, *Bacillus marisflavi* and *Bacillus oshimensis*. The species of the genus *Oceanobacillus* were mainly *Oceanobacillus oncorhynchi* subsp. *incaldanensis* and *Oceanobacillus kimchii*. The species of the genus *Virgibacillus* were mainly *Virgibacillus dokdonensis* and *Virgibacillus halodenitrificans*.

The species of the phylum Proteobacteria mainly belonged to the genera *Pseudomonas*, *Psychrobacter*, *Halomonas* and *Pseudoalteromonas*. There were no isolates affiliated to members of the genus *Vibrio*. The species of the phylum Actinobacteria belonged to the genera *Nocardiopsis*, *Streptomyces* and *Williamsia*. The closest relatives of these isolates were observed in various locations including coastal environments, sea animals, soil, etc. A few strains were isolated from the seawater sample in this research and they belonged with genera *Psychrobacter*, *Pseudomonas*

and *Williamsia* (actinobacteria).

Table 2 showed pairwise comparisons of species compositions between different size groups of the sea cucumber and between the sea cucumber and sea sediment as expressed by the Sørensen index. The highest value was observed between the adult and the small sea cucumber groups. Values of similarity index for the sea sediment increased as the body size of sea cucumber increased. Similarity index for comparison between black and green groups was 0.568 and there was no clear difference between them.

Twenty-seven isolates (in Supplementary Table 2 Web site) showed less than 97% identities with any type strain sequences, suggesting that these isolates were new species or new genus. Among the 27 isolates, I found 6 tentative species defined with "97% sequence identity (607-613bp of partial 16S rRNA gene sequence). It was worth noting that almost all (26 out of 27) isolates were obtained from alkaline agar plates (pH 10.3-10.5) and 23 isolates were found on the plates containing 10% NaCl. Four tentative species (tentative species 1: isolates U0063, U0071, U0112, U0179, U0195, U0204, U0211, U0241, U0281, U0378; tentative species 2: isolate U0217; tentative species 3: isolate U0557; tentative species 4: U1120) were found only in the intestine of sea cucumbers and 2 tentative species (tentative species 5: isolates U0034, U0038, U0062, U0094, U0137, U0147, U0167, U0205, U0326, U0377; tentative species 6: isolates U0087, U0100, U0142, U0320) were found in both the intestine and the sea sediment.

Polysaccharide degradation ability of isolates

The 231 isolates from various samples showed various polysaccharides

degradation ability and degraded one or more substrates (S, CMC, AL or XL). Twenty-seven, 14 and 14 species from the intestines showed amylase activity, cellulase activity and xylanase activity, respectively (Fig.2, Supplementary Table 2 Web site). There were no isolates showing alginate or agar degradation activities. On the other hand, 18 species from the intestines had no activity to degrade these polysaccharides. Most of the species showing various polysaccharides degradation activities belonged to the families Bacillaceae 1 and 2 (Fig.2). The bacterial diversity of polysaccharide degrading isolates was almost similar among samples from the 6 kinds of sea cucumbers and the sea sediment except xylan degradation activity. The species showing xylan degradation were detected in the intestines but few in the sediment (Fig.2).

Amylase producing isolates were mainly affiliated with the genus *Bacillus*, namely *Bacillus amyloliquefaciens*, *Bacillus aryabhatai*, *Bacillus clausii*, *Bacillus hunanensis*, *Bacillus licheniformis*, *Bacillus oshimensis* and *Bacillus subtilis*. The majority of cellulase positive isolates were affiliated to *Virgibacillus dokdonensis*, *Bacillus hunanensis* and *Bacillus oshimensis*. The xylanase positive isolates were mainly *Bacillus stratosphericus* / *Bacillus aerophilus* / *Bacillus altitudinis* group, *Bacillus pumilus*/*Bacillus safensis* group, *Bacillus subtilis*. The isolates of *Geomicrobium halophilum*, *Virgibacillus halodenitrificans* and *Virgibacillus marismortui* showed no polysaccharide degradation ability.

Physiological characteristics of the isolates

Fig. 3 and Supplementary Table 2 (Web site) showed effect of anaerobic condition for growth of the isolates. The 231 isolates from various samples were

divided into three groups, facultative anaerobic bacteria (FA), anaerobic tolerant bacteria (AT) and aerobic bacteria (A). Diversity of FA, AT and A groups was similar between the intestines and the sea sediment and most of the isolates belonged to the families Bacillaceae 1 and 2 (Fig.3). Facultative anaerobic isolates were mainly affiliated with *Virgibacillus dokdonensis*, *Bacillus licheniformis*, *Bacillus aerophilus* / *Bacillus altitudinis* / *Bacillus stratosphericus* group and *Oceanobacillus oncorhynchi* subsp. *incaldanensis*.

Anaerobic tolerant isolates were mainly affiliated with *Bacillus clausii*, *Bacillus hunanensis*, *Bacillus oshimensis*, *Bacillus marisflavi*, *Geomicrobium halophilum*, *Virgibacillus halodenitrificans*, *Oceanobacillus kimuchii* and *Pseudomonas gesardii*. Aerobic isolates were affiliated with the genera *Bacillus*, *Halobacillus* and *Pseudomonas*.

Salinity tolerance of the isolates was examined (Supplementary Table 2 Web site). Eleven isolates were halophilic ($\geq 25\%$ NaCl conc.) and belonged to the genera *Halobacillus*, *Virgibacillus* and *Oceanobacillus*. Most of isolates showing 20-25 % NaCl tolerance belonged to the family Bacillaceae 2 such as the genera *Halobacillus*, *Virgibacillus* and *Oceanobacillus*. On the other hand, most isolates showing 10-15 % NaCl tolerance belonged to the genera *Bacillus*, *Geomicrobium* and *Pseudomonas*. It appears that the strains isolated from 3.5 % NaCl plates showed 10-15 % salinity tolerance and the strains isolated from 10 % NaCl plates showed 15-20 % salinity tolerance (Supplementary Table 2 Web site). The salinity tolerance of the isolates was similar among samples from the intestines and the sea sediment.

All isolates (231 strains) were examined for growth responses to pH shift (pH7→pH10 or pH10→pH7) (Supplementary Table 2 Web site). All alkaliphilic strains isolated from alkali medium were able to grow at pH 7, and more than half isolates from pH 7 were able to grow at pH 10. All neutrophiles were mainly affiliated with the family Bacillaceae 1 such as *Bacillus amyloliquefaciens*, *Bacillus aryabhatai* and *Bacillus subtilis*. The isolates belonging to the family Bacillaceae 2 and more than half of the isolates belonging to the family Bacillaceae 1 were alkaliphiles.

3.4 Discussion

In this report, I isolated various aerobic culturable bacteria from the guts of *Apostichopus japonicus*. Analysis of partial 16S rRNA gene sequences of 231 isolates indicated that they were classified into 53 species in the families Bacillaceae 1 and 2 of the phylum Firmicutes, the class Gammaproteobacteria and the phylum Actinobacteria. High diversity was observed in the genus *Bacillus* (20 species), *Oceanobacillus* (6 species) and *Virgibacillus* (4 species). The isolated species were often observed in sea environments, sea animals and the Far East area. Most isolates showed salt-tolerance and alkaliphilic properties, suggesting that these isolates were derived from sea environment.

Microbial diversity was almost similar among the samples of adult, small, juvenile sea cucumbers and also among the samples of black and green sea cucumbers. Moreover, a substantial number of bacterial species were found to be common between

the holothurians gut and the sea sediment. In contrast to my culture-dependent method, Amaro et al. performed culture-independent methods and reported that the gut bacterial composition of the abyssal holothurian *Molpadia musculus* was similar to that of the organic matter-rich sediments (Amaro et al., 2009). Recently, they also found that ca. 82% of total bacterial OTUs were common between the gut contents and the surrounding sediments (Amaro et al., 2010).

Surprisingly, there were no isolates affiliated to members of the genus *Vibrio* among various samples of *Apostichopus japonicus*, the sea sediment and seawater collected in this research. On the other hand, Enomoto et al. reported that Gammaproteobacteria members including *Vibrio* spp. were isolated as culturable bacteria from the intestine of *Apostichopus japonicus* (Enomoto et al., 2012). It was reported that the frequency and level of *Vibrio* species were much lower during winter than summer months (Chowdhury et al., 1990; Colwell, 1979). *Vibrio* species were well known pathogens for sea animals (Austin, 2010). The seawater temperature of the open sea near Nagasaki area was ca.15°C in Jan. 2011 (Data from Japan Meteorological Agency). But Omura bay was inland bay and the seawater temperature was less than 10°C in winter, ca.5 degree lower than the open seawater temperature near Nagasaki. Omura Bay is known as production area of sea cucumbers since Edo era. Probably this low temperature in winter contributes to the production of healthy sea cucumbers in Omura bay.

Detritus was a source of nutrient for detritus feeders and bacteria were the main decomposers that degrade these materials (Hagen, et al., 2012). Therefore, I analyzed

polysaccharide degradation of the isolates. Most isolates showed starch, CMC or xylan degradation abilities but few isolates were able to degrade alginate or agar. On the other hand, most isolates were facultative anaerobic bacteria or anaerobic tolerant bacteria, indicating that most isolates were alive in the intestine of the sea cucumber. Although there has never been convincing evidence for intestinal environments of sea cucumbers, it is highly probable that oxygen will enter the intestine of the sea cucumber from the mouth with the detritus food and also some amount can penetrate from the body tissues. Our results suggested that the aerobic culturable isolates in this study potentially contributed to digest detritus and supply fermentation products (minor components and vitamins) to their host sea cucumber, although it is yet unclear whether aerobic isolates obtained in this study are permanent residents in the intestines or not.

High salt concentration or high pH were used for isolation conditions to isolate various bacteria because marine water is semi-alkaline pH and contains 3.5% NaCl. Most of isolates showing 20-25 % NaCl tolerance belonged to the family Bacillaceae 2 such as the genera *Halobacillus*, *Virgibacillus* and *Oceanobacillus*. Isolates belonging to the family Bacillaceae 2 and more than half of isolates belonging to the family Bacillaceae 1 were alkaliphiles. On the other hand, 27 isolates (Supplementary Table 2 Web site) showed less than 97% identities with any type strain sequences. These isolates were classified into 6 groups with $\geq 97\%$ sequence identity (607-613bp of partial 16S rRNA gene sequence). Most of them were alkaliphiles obtained from plates at pH10 and 10 % NaCl. These results suggested that the intestines of holothurians were resources for new species.

Many isolates classified into the species as mentioned below were reported to have denitrification ability: *Virgibacillus halodenitrificans* (Denariáz et al., 1989), *Oceanobacillus oncorhynchi* subsp. *incaldanensis* (Raats and Halpern, 2007), *Pseudomonas gessardii* (Verhille et al., 1999), *Pseudovibrio japonicus* (Hosoya and Yokota, 2007), *Gracilibacillus dipsosauri* (Lawson et al., 1996) and *Oceanobacillus chironomi* (Raats and Halpern, 2007). Probably these isolates play an important role to denitrify nitrate derived from human sewage from cities surrounding Omura bay but the role in the intestine was not clear.

Although culture-dependent approaches limits our ability to quantify prokaryotic diversity in the holothurian gut, I believe that physiological examinations of isolated bacteria contribute to further understanding of invertebrate-microbe interactions in combination with metagenomic approaches.

3.5 Summary

Various aerobic culturable bacteria (1133 isolates) were isolated from the gut of *Apostichopus japonicus* (black adult, green adult, black small, green small, black juvenile, and green juvenile sea cucumbers) and from the sea sediment and the seawater using different culture conditions and without enrichment culture. By molecular analysis of partial 16S rRNA gene sequences of 231 isolates, they were tentatively affiliated with 53 described species in the phyla Firmicutes (42 species), Proteobacteria (9 species) and Actinobacteria (2 species). Eighteen species were often found among the

intestines and the sea sediment. High diversity was observed in the genus *Bacillus* (20 species), *Oceanobacillus* and *Virgibacillus* but there were no isolates affiliated to members of the genus *Vibrio*, well-known sea pathogens. There were no clear differences in the bacterial communities among the hosts varied in size and color. Most isolates showed various polysaccharide degradation activities, suggesting their possible contributions in the digestion of organic matters in the gut.

Table 1 Phylogenetic affiliation for isolates (231 strains) from various specimens

phylum/class/family	genus	species /tentative species	specimens								number of specimens
			①	③	⑤	⑥	⑦	⑧	⑨	⑩	
phylum Firmicutes											
family Bacillaceae 1	<i>Bacillus</i> (20)	<i>Bacillus aerophilus</i> / <i>Bacillus altitudinis</i> / <i>Bacillus stratosphericus</i> *	+	+	+	+	+	+	+	+	7
		<i>Bacillus amyloliquefaciens</i> *	+	+							3
		<i>Bacillus aquimaris</i>	+		+						2
		<i>Bacillus aryabhatai</i> *	+	+	+	+	+	+	+	+	6
		<i>Bacillus cereus</i>						+			1
		<i>Bacillus clarkii</i> / <i>Bacillus polygoni</i>	+	+							2
		<i>Bacillus clausii</i> *	+	+	+	+	+	+	+	+	7
		<i>Bacillus farraginis</i>						+			1
		<i>Bacillus firmus</i>				+				+	2
		<i>Bacillus gibsonii</i>	+								1
		<i>Bacillus horikoshii</i>							+		1
		<i>Bacillus hunanensis</i> *	+	+	+	+	+	+	+	+	7
		<i>Bacillus hunanensis</i> / <i>Bacillus lehensis</i>	+	+							2
		<i>Bacillus krulwichiae</i>								+	1
		<i>Bacillus licheniformis</i> *	+	+	+	+	+	+	+	+	7
		<i>Bacillus marisflavi</i> *	+	+	+	+	+	+	+	+	5
		<i>Bacillus methylotrophicus</i>				+					1
		<i>Bacillus okhensis</i> / <i>Bacillus wakoensis</i>	+								1
		<i>Bacillus okhensis</i> / <i>Bacillus krulwichiae</i>					+				1
		<i>Bacillus oshimensis</i> *	+	+	+						3
		<i>Bacillus polygoni</i>						+			1
		<i>Bacillus pseudofirmus</i>	+	+							2
		<i>Bacillus pumilus</i>	+								1
		<i>Bacillus pumilus</i> / <i>Bacillus safensis</i> *	+	+						+	3
		<i>Bacillus subtilis</i>				+	+				2
		<i>Bacillus vietnamensis</i>			+						1
family Bacillaceae 2	<i>Filobacillus</i> (1)	<i>Filobacillus milensis</i>								+	1
family Bacillaceae 2	<i>Geomicrobium</i> (1)	<i>Geomicrobium halophilum</i> *	+	+	+	+	+	+	+	+	6
family Bacillaceae 2	<i>Gracilibacillus</i> (3)	<i>Gracilibacillus dipsosauri</i>	+								1
		<i>Gracilibacillus halotolerans</i>					+				1
		<i>Gracilibacillus saliphilus</i>	+								1
family Bacillaceae 2	<i>Halobacillus</i> (2)	<i>Halobacillus kuroshimensis</i> *	+	+	+						3
		<i>Halobacillus trueperi</i>				+					1
		<i>Halobacillus yeomyeoni</i> / <i>Halobacillus trueperi</i> / <i>Halobacillus litoralis</i>	+								1
family Bacillaceae 2	<i>Halolactibacillus</i> (1)	<i>Halolactibacillus alkaphilus</i>			+						1
family Bacillaceae 2	<i>Oceanobacillus</i> (6)	<i>Oceanobacillus chironomi</i>					+				1
		<i>Oceanobacillus oncorhynchi</i> subsp. <i>incaldanensis</i> *	+	+	+	+	+	+	+	+	5
		<i>Oceanobacillus kimchii</i> *	+				+			+	3
		<i>Oceanobacillus picturae</i>	+							+	2
		<i>Oceanobacillus profundus</i>								+	1
		<i>Oceanobacillus sojae</i>				+					1
family Bacillaceae 2	<i>Salsuginibacillus</i> (1)	<i>Salsuginibacillus kocurii</i>	+				+				2
family Bacillaceae 2	<i>Virgibacillus</i> (4)	<i>Virgibacillus dokdonensis</i> *	+	+	+	+	+	+	+	+	7
		<i>Virgibacillus chiguensis</i>			+						1
		<i>Virgibacillus halodenitrificans</i> *	+	+	+	+	+	+	+	+	6
		<i>Virgibacillus marismortui</i> *	+	+	+						3
		<i>Virgibacillus marismortui</i> / <i>Virgibacillus salarius</i> *	+	+	+	+	+	+	+	+	6
family Planococcaceae	<i>Lysinibacillus</i> (1)	<i>Lysinibacillus fusiformis</i>					+	+			2
family Planococcaceae	<i>Planococcus</i> (1)	<i>Planococcus maritimus</i>								+	1
family Planococcaceae	<i>Sporosarcina</i> (1)	<i>Sporosarcina saromensis</i>	+								1
phylum Proteobacteria											
class alpha	<i>Pseudovibrio</i> (1)	<i>Pseudovibrio japonicus</i>						+			1
class gamma	<i>Ferrimonas</i> (1)	<i>Ferrimonas senticii</i>						+			1
class gamma	<i>Halomonas</i> (1)	<i>Halomonas meridiana</i>								+	1
class gamma	<i>Pseudomonas</i> (3)	<i>Pseudomonas cedrina</i> subsp. <i>fulgida</i>					+	+			2
		<i>Pseudomonas gessardii</i> *	+						+	+	3
		<i>Pseudomonas libaniensis</i>								+	1
class gamma	<i>Pseudoalteromonas</i> (1)	<i>Pseudoalteromonas tetraodonis</i>						+			1
class gamma	<i>Psychrobacter</i> (2)	<i>Psychrobacter celer</i>							+		1
		<i>Psychrobacter nivimaris</i>							+		1
phylum Actinobacteria											
family Nocardiopsaceae	<i>Nocardiopsis</i> (1)	<i>Nocardiopsis lucentensis</i>	+								1
family Streptomycetaceae	<i>Streptomyces</i> (1)	<i>Streptomyces gougerotii</i> / <i>Streptomyces rutgersensis</i>						+			1
family Williamsiaceae	<i>Williamsia</i> (1)	<i>Williamsia serinedens</i>							+		1
		number of nearest type strain species	16	26	17	20	20	20	4	21	

① ~ ⑧ indicated samples from black adult ①, green adult ③, black small ⑤, green small ⑥, black juvenile ⑦, and green juvenile ⑧ sea cucumbers, respectively. ⑨ and ⑩ indicated samples from seawater ⑨ and sea sediment ⑩, respectively. + indicated presence of species. () indicated number of the species. * indicated the species found in more than 3 samples. Display of more than one species in the column of species indicated the same identity in the comparison range.

Table 2. Similarity indices for the different size groups of sea cumpers (adult, small and juvenile) and the sea sediment				
Samples	Adult	Small	Juvenile	Sediment
Adult				
Small	0.633			
Juvenile	0.467	0.500		
Sediment	0.528	0.449	0.408	



Fig.1 Map of sampling site and environmental conditions.

A

Sampling point : 32°53'N, 129°57'E
(near Kushima, Omura bay, Nagasaki, Japan)

Sampling data: 2011.1.28

Surface water temperature: 7.4°C

Salinity of surface water 2.85%

Water depth of samples : 4 m

Temperature: 7.1 °C

Salinity: 2.93%

Reference data

Open sea surface water temperature: 13-15°C
(Data from Japan Meteorological Agency)

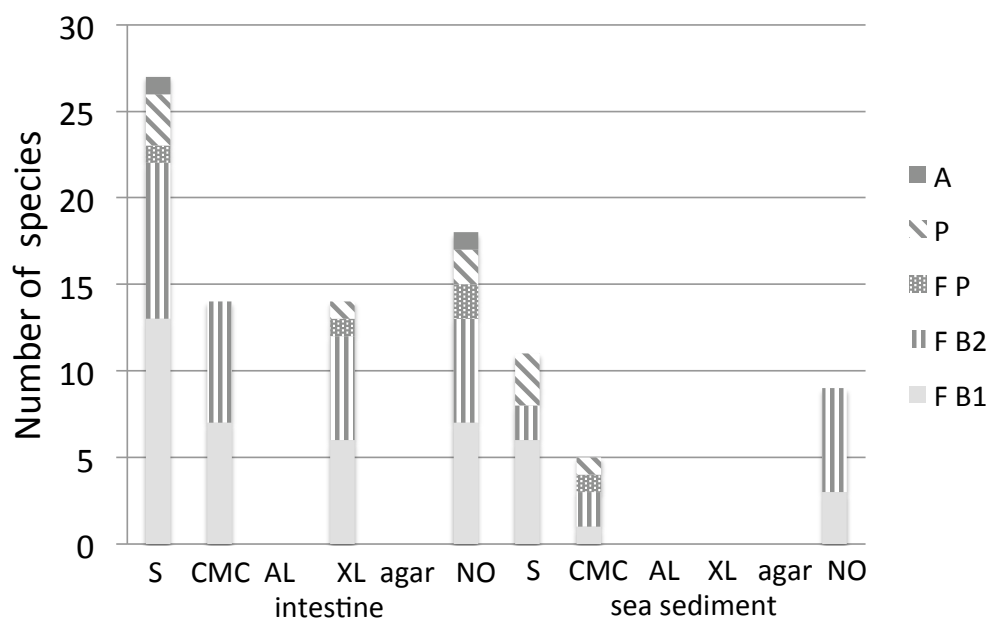


Fig.2 Polysaccharide degradation ability of isolates S, CMC, AL, XL, agar and NO indicated starch degradation activity, CMC degradation activity, alginate degradation activity, xylan degradation activity, agar degradation activity and No degradation activity of polysaccharides mentioned in this study, respectively. Gray box (FB1): the phylum Firmicutes, the family Bacillaceae 1, Vertical stripes (FB2): the phylum Firmicutes, the family Bacillaceae 2, Dotted box (FP): the phylum Firmicutes, the family Planococcaceae, Diagonal stripes box (P): the phylum Proteobacteria, Black box (A): the phylum Actinobacteria. Fig.2 was summarized from Supplementary Table 2.

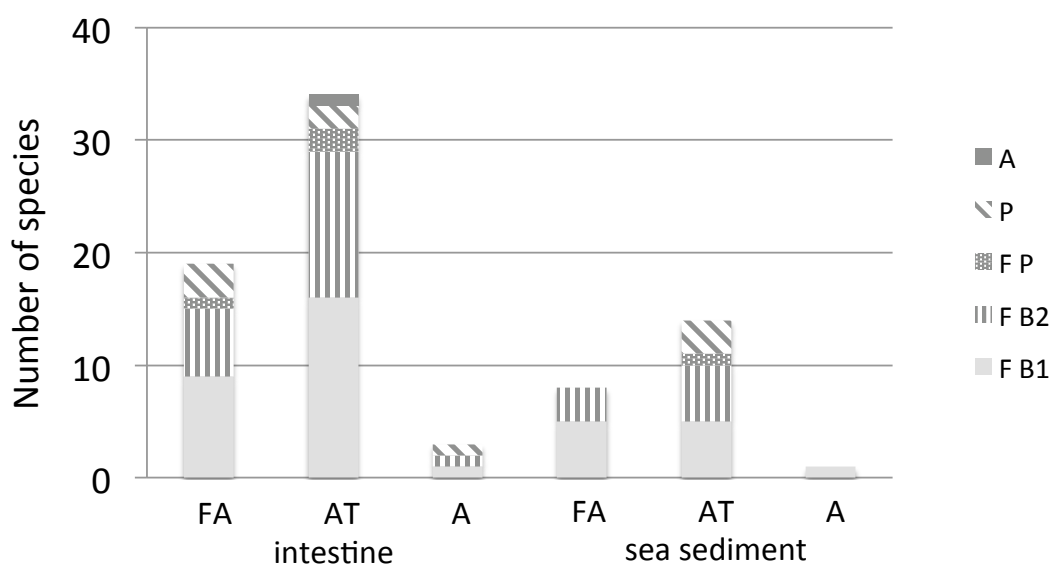


Fig.3 Effect of oxygen on the growth of isolates

Facultative anaerobic bacteria (FA) form colony in both aerobic and anaerobic cultivation. Anaerobic tolerant bacteria (AT) do not form colony in anaerobic condition for two weeks but form colony in aerobic cultivation after the anaerobic cultivation. Aerobic bacteria (A) do not form colony in anaerobic condition for two weeks and also do not form colony in aerobic cultivation after the anaerobic cultivation.

Gray box (FB1): the phylum Firmicutes, the family Bacillaceae 1, Vertical stripes (FB2): the phylum Firmicutes, the family Bacillaceae 2, Dotted box (FP): the phylum Firmicutes, the family Planococcaceae, Diagonal stripes box (P): the phylum Proteobacteria, Black box (A): the phylum Actinobacteria. Fig.3 was summarized from Supplementary Table 2.

Supplementary table1 The number of the isolates obtained by different culture conditions

Isolation condition		①black adult	③green adult	⑤ black small	⑥green small	⑦black juvenile	⑧green juvenile	⑨seawater	⑩sea sediment	number of isolates
pH 10,10%	CMC	25	19	9	12	7	12	0	13	97
	AL	12	12	8	8	10	6	0	11	67
	XL	13	14	13	9	10	8	0	6	73
	S	18	19	8	11	11	13	0	9	89
pH 10,3.5%	CMC	12	14	8	7	7	5	0	7	60
	AL	7	7	2	4	2	2	0	2	26
	XL	14	22	13	12	7	10	0	8	86
	S	16	18	10	9	9	8	0	9	79
subtotal		117	125	71	72	63	64	0	65	577
pH 7,10%	CMC	15	9	12	10	5	10	1	10	72
	AL	11	14	14	11	11	7	0	8	76
	XL	10	21	12	9	9	7	0	12	80
	S	23	21	7	8	14	10	0	5	88
pH 7,3.5%	CMC	11	13	7	7	4	5	1	6	54
	AL	6	6	4	2	3	2	0	3	26
	XL	17	12	4	6	7	7	1	6	60
	S	15	9	5	7	5	5	0	7	53
	LB	12	10	3	6	6	4	1	5	47
subtotal		120	115	68	66	64	57	4	62	556
total number of isolates		237	240	139	138	127	121	4	127	1133

Supplementary Table 2

Isolate no.	Accession number of isolates	Isolation medium	Specimen Number	species / tentative species	Accession number of species / tentative species	Identities	Degrading activities on polysaccharides				Requirement of oxygen	Maximum NaCl concentration for growth	pH tolerance	phylum	family
							S	CMC	AI	XL					
U0211	AB734880	XL(pH10.10%)	⑥	<i>Virgibacillus marismortui</i>	AJ009793	796/828 (96%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0217	AB734881	XL(pH10.10%)	⑦	<i>Virgibacillus halodentirificans</i>	AY543169	677/705 (96%), Gaps = 1/705 (0%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0229	AB734882	XL(pH10.10%)	⑧	<i>Virgibacillus dokdonensis</i>	AY822043	607/609 (99%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0230	AB734883	XL(pH10.10%)	⑧	<i>Bacillus humanensis</i>	HM054473	795/819 (97%), Gaps = 2/819 (0%)	+	-	-	+	AT	10	ALK	firmicutes	family Bacillaceae 1
U0241	AB734884	S(pH10.10%)	①	<i>Virgibacillus marismortui</i> / <i>Virgibacillus salarius</i>	AJ009793/AB197851	649/679 (95%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0260	AB734885	S(pH10.10%)	③	<i>Virgibacillus dokdonensis</i>	AY822043	582/587 (99%), Gaps = 1/587 (0%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0262	AB734886	S(pH10.10%)	③	<i>Bacillus okhensis</i> / <i>Bacillus wakoensis</i>	DQ026060/AB043851	638/646 (98%)	-	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U0275	AB734887	S(pH10.10%)	⑤	<i>Virgibacillus dokdonensis</i>	AY822043	718/720 (99%), Gaps = 1/720 (0%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0277	AB734888	S(pH10.10%)	⑤	<i>Virgibacillus dokdonensis</i>	AY822043	564/569 (99%), Gaps = 1/569 (0%)	-	+	-	-	FA	25	ALK	firmicutes	family Bacillaceae 2
U0279	AB734889	S(pH10.10%)	⑤	<i>Bacillus clarckii</i> / <i>Bacillus polygami</i>	X76444/AB292819	529/531 (99%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 1
U0281	AB734890	S(pH10.10%)	⑤	<i>Virgibacillus marismortui</i> / <i>Virgibacillus salarius</i>	AJ009793/AB197851	758/783 (96%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0285	AB734891	S(pH10.10%)	⑥	<i>Bacillus humanensis</i>	HM054473	795/819 (97%), Gaps = 2/819 (0%)	+	+	-	+	AT	15	ALK	firmicutes	family Bacillaceae 1
U0286	AB734892	S(pH10.10%)	⑥	<i>Virgibacillus dokdonensis</i>	AY822043	660/662 (99%), Gaps = 1/662 (0%)	+	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0294	AB734893	S(pH10.10%)	⑦	<i>Bacillus clausii</i>	X76440	639/640 (99%)	+	+	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0295	AB734894	S(pH10.10%)	⑦	<i>Oceanobacillus oncorhynchi subsp. incaldanensis</i>	AJ640134	672/672 (100%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0299	AB734895	S(pH10.10%)	⑦	<i>Gracilbacillus halotolerans</i>	AJF036922	590/595 (99%)	+	+	-	+	AT	15	ALK	firmicutes	family Bacillaceae 2
U0302	AB734896	S(pH10.10%)	⑦	<i>Virgibacillus dokdonensis</i>	AY822043	697/700 (99%), Gaps = 1/700 (0%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0304	AB734897	S(pH10.10%)	⑦	<i>Virgibacillus dokdonensis</i>	AY822043	658/660 (99%)	+	+	-	-	FA	25	ALK	firmicutes	family Bacillaceae 2
U0305	AB734898	S(pH10.10%)	⑧	<i>Bacillus polygami</i>	AB292819	660/674 (97%), Gaps = 1/674 (0%)	-	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U0308	AB734899	S(pH10.10%)	⑧	<i>Virgibacillus dokdonensis</i>	AY822043	708/711 (99%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0309	AB734900	S(pH10.10%)	⑧	<i>Bacillus humanensis</i>	HM054473	817/818 (99%)	+	+	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0316	AB734901	S(pH10.10%)	⑧	<i>Bacillus horikoshii</i>	AB043865	818/820 (99%), Gaps = 1/820 (0%)	-	+	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U0318	AB734902	S(pH10.10%)	⑩	<i>Virgibacillus dokdonensis</i>	AY822043	500/501 (99%)	+	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0320	AB734903	S(pH10.10%)	⑩	<i>Geomicrobium halophilum</i>	AB449106	745/821 (90%), Gaps = 2/821 (0%)	+	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0326	AB734904	S(pH10.10%)	⑩	<i>Bacillus firmus</i>	I16268	570/610 (93%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0329	AB734905	CMC(pH10.3.5%)	①	<i>Virgibacillus dokdonensis</i>	AY822043	807/808 (99%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0377	AB734906	CMC(pH10.3.5%)	⑧	<i>Geomicrobium halophilum</i>	AB449106	724/788 (91%), Gaps = 1/788 (0%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 2
U0378	AB734907	CMC(pH10.3.5%)	⑧	<i>Virgibacillus marismortui</i> / <i>Virgibacillus salarius</i>	AJ009793/AB197851	734/759 (96%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0449	AB734908	XL(pH10.3.5%)	⑤	<i>Halotacibacillus alkaliphilus</i>	EFS54593	463/473 (97%)	-	-	-	+	FA	10	ALK	firmicutes	family Bacillaceae 2
U0451	AB734909	XL(pH10.3.5%)	⑤	<i>Virgibacillus dokdonensis</i>	AY822043	703/705 (99%), Gaps = 1/705 (0%)	+	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0494	AB734910	XL(pH10.3.5%)	⑩	<i>Bacillus krubwickiae</i>	AB086897	739/739 (100%)	+	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 1
U0534	AB734911	S(pH10.3.5%)	⑤	<i>Bacillus clausii</i>	X76440	584/586 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U0557	AB734912	S(pH10.3.5%)	⑦	<i>Bacillus okhensis</i> / <i>Bacillus krubwickiae</i>	DQ026060/AB086897	746/776 (96%), Gaps = 1/776 (0%)	-	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 1
U0561	AB734913	S(pH10.3.5%)	⑧	<i>Bacillus clausii</i>	X76440	552/553 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U0564	AB734914	S(pH10.3.5%)	⑧	<i>Bacillus humanensis</i>	HM054473	644/644 (100%)	+	+	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0565	AB734915	S(pH10.3.5%)	⑧	<i>Bacillus humanensis</i>	HM054473	796/820 (97%), Gaps = 2/820 (0%)	+	+	-	+	AT	15	ALK	firmicutes	family Bacillaceae 1
U0567	AB734916	S(pH10.3.5%)	⑧	<i>Virgibacillus dokdonensis</i>	AY822043	662/663 (99%)	-	+	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0580	AB734917	CMC(pH7.10%)	③	<i>Halobacillus kuroshimensis</i>	AB195680	752/755 (99%), Gaps = 2/755 (0%)	+	-	-	-	A	20	NE	firmicutes	family Bacillaceae 2
U0593	AB734918	CMC(pH7.10%)	①	<i>Bacillus humanensis</i> / <i>Bacillus lehensis</i>	HM054473/AY793550	660/662 (99%)	+	+	-	-	A	15	ALK	firmicutes	family Bacillaceae 1
U0611	AB734919	CMC(pH7.10%)	⑤	<i>Virgibacillus dokdonensis</i>	AY822043	784/785 (99%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0613	AB734920	CMC(pH7.10%)	⑤	<i>Virgibacillus dokdonensis</i>	AY822043	758/763 (99%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0614	AB734921	CMC(pH7.10%)	⑥	<i>Oceanobacillus sojiae</i>	AB473561	762/775 (98%)	+	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0615	AB734922	CMC(pH7.10%)	⑥	<i>Virgibacillus halodentirificans</i>	AY543169	745/747 (99%)	-	-	-	-	AT	25	ALK	firmicutes	family Bacillaceae 2
U0627	AB734923	CMC(pH7.10%)	⑦	<i>Virgibacillus dokdonensis</i>	AY822043	724/726 (99%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0639	AB734924	CMC(pH7.10%)	⑨	<i>Pseudomonas gessardii</i>	AF074384	729/732 (99%)	-	-	-	-	AT	10	NE	proteobacteria	gamma
U0653	AB734925	AL(pH7.10%)	①	<i>Gracilbacillus diposauroi</i>	AB101591	764/764 (100%)	+	-	-	+	AT	15	NE	firmicutes	family Bacillaceae 2
U0665	AB734926	AL(pH7.10%)	③	<i>Virgibacillus halodentirificans</i>	AY543169	795/797 (99%), Gaps = 1/797 (0%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0683	AB734927	AL(pH7.10%)	⑤	<i>Halobacillus kuroshimensis</i>	AB195680	794/798 (99%), Gaps = 2/798 (0%)	+	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 2
U0684	AB734928	AL(pH7.10%)	⑤	<i>Bacillus humanensis</i> / <i>Bacillus lehensis</i>	HM054473/AY793550	610/611 (99%)	+	+	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
U0685	AB734929	AL(pH7.10%)	⑤	<i>Bacillus vietnamensis</i>	AB099708	652/658 (99%), Gaps = 1/658 (0%)	+	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
U0737	AB734930	XL(pH7.10%)	③	<i>Virgibacillus halodentirificans</i>	AY543169	780/780 (100%)	-	-	-	-	AT	25	ALK	firmicutes	family Bacillaceae 2
U0738	AB734931	XL(pH7.10%)	③	<i>Pseudomonas gessardii</i>	AJF074384	632/635 (99%)	+	-	-	-	AT	10	NE	proteobacteria	gamma
U0757	AB734932	XL(pH7.10%)	⑤	<i>Bacillus licheniformis</i>	CP000002	822/829 (99%), Gaps = 1/829 (0%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U0760	AB734933	XL(pH7.10%)	⑤	<i>Virgibacillus halodentirificans</i>	AY543169	784/787 (99%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0761	AB734934	XL(pH7.10%)	⑤	<i>Halobacillus kuroshimensis</i>	AB195680	835/842 (99%), Gaps = 2/842 (0%)	+	-	-	-	AT	25	NE	firmicutes	family Bacillaceae 2
U0766	AB734935	XL(pH7.10%)	⑤	<i>Virgibacillus dokdonensis</i>	AY822043	804/809 (99%), Gaps = 1/809 (0%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0767	AB734936	XL(pH7.10%)	⑤	<i>Virgibacillus halodentirificans</i>	AY543169	807/809 (99%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U0770	AB734937	XL(pH7.10%)	⑥	<i>Halobacillus truegeri</i>	AJ310149	760/762 (99%)	+	-	-	-	AT	25	ALK	firmicutes	family Bacillaceae 2
U0774	AB734938	XL(pH7.10%)	⑥	<i>Virgibacillus halodentirificans</i>	AY543169	691/694 (99%), Gaps = 2/694 (0%)	-	-	-	-	AT	25	ALK	firmicutes	family Bacillaceae 2
U0784	AB734939	XL(pH7.10%)	⑦	<i>Virgibacillus halodentirificans</i>	AY543169	694/694 (100%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0830	AB734940	S(pH7.10%)	③	<i>Bacillus licheniformis</i>	CP000002	814/819 (99%)	+	-	-	-	FA	10	NE	firmicutes	family Bacillaceae 1
U0834	AB734941	S(pH7.10%)	③	<i>Halobacillus kuroshimensis</i>	AB195680	801/805 (99%), Gaps = 2/805 (0%)	+	-	-	+	AT	10	NE	firmicutes	family Bacillaceae 1
U0835	AB734942	S(pH7.10%)	③	<i>Oceanobacillus picturae</i>	AJ315060	756/757 (99%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0860	AB734943	S(pH7.10%)	⑥	<i>Bacillus licheniformis</i>	CP000002	820/821 (99%), Gaps = 1/821 (0%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1

Supplementary Table 2

Isolate no.	Accession number of isolates	Isolation medium	Specimen Number	species / tentative species	Accession number of species / tentative species	Identities	Degrading activities on polysaccharides				Requirement of oxygen	Maximum NaCl concentration for growth	pH tolerance	phylum	family
							S	CMC	AI	XL					
U0892	AB734944	SlpH7.10%	⑩	<i>Pseudomonas gessardii</i>	AF074384	747/750 (99%)	+	-	-	-	AT	15	ALK	proteobacteria	gamma
U0893	AB734945	SlpH7.10%	⑩	<i>Bacillus licheniformis</i>	CP000002	816/820 (99%), Gaps = 1/820 (0%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0894	AB734946	CMC(pH7.3.5%)	①	<i>Bacillus stratosphericus</i> / <i>Bacillus aerophilus</i> / <i>Bacillus altitudinis</i>	AJ831841/AJ831844/AJ831842	768/769 (99%), Gaps = 1/769 (0%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U0895	AB734947	CMC(pH7.3.5%)	①	<i>Bacillus aquimaris</i>	AF483625	683/695 (98%)	+	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
U0898	AB734948	CMC(pH7.3.5%)	①	<i>Bacillus clausii</i>	X76440	803/813 (98%), Gaps = 2/813 (0%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0899	AB734949	CMC(pH7.3.5%)	①	<i>Bacillus clausii</i>	X76440	804/813 (98%), Gaps = 2/813 (0%)	-	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U0905	AB734950	CMC(pH7.3.5%)	③	<i>Bacillus pumilus</i> / <i>Bacillus safensis</i>	AY876289/AF234854	807/807 (100%)	-	-	-	+	AT	15	ALK	firmicutes	family Bacillaceae 1
U0906	AB734951	CMC(pH7.3.5%)	③	<i>Bacillus marisflavi</i>	AF483624	629/635 (99%), Gaps = 1/635 (0%)	+	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
U0907	AB734952	CMC(pH7.3.5%)	③	<i>Sporosarcina saromensis</i>	AB243859	782/785 (99%)	-	-	-	-	AT	10	ALK	firmicutes	family Planococcaceae
U0908	AB734953	CMC(pH7.3.5%)	③	<i>Bacillus altitudinis</i> / <i>Bacillus stratosphericus</i>	AJ831842/AJ831841	818/818 (100%)	-	-	-	+	AT	10	ALK	firmicutes	family Bacillaceae 1
U0923	AB734954	CMC(pH7.3.5%)	⑤	<i>Virgibacillus halodentificans</i>	AY543169	842/845 (99%), Gaps = 2/845 (0%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0924	AB734955	CMC(pH7.3.5%)	⑤	<i>Bacillus clausii</i>	X76440	738/742 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0925	AB734956	CMC(pH7.3.5%)	⑤	<i>Bacillus aquimaris</i>	AF483625	743/756 (98%)	+	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 1
U0926	AB734957	CMC(pH7.3.5%)	⑤	<i>Pseudomonas cedrina</i> subsp. <i>fulgida</i>	AJ492830	552/553 (99%)	+	-	-	-	AT	15	ALK	proteobacteria	gamma
U0927	AB734958	CMC(pH7.3.5%)	⑤	<i>Bacillus aryabhatai</i>	EF114313	809/810 (99%)	+	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
U0928	AB734959	CMC(pH7.3.5%)	⑤	<i>Virgibacillus halodentificans</i>	AY543169	779/781 (99%), Gaps = 1/781 (0%)	+	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0932	AB734960	CMC(pH7.3.5%)	⑦	<i>Pseudomonas cedrina</i> subsp. <i>fulgida</i>	AJ492830	551/552 (99%)	+	-	-	-	A	10	ALK	proteobacteria	gamma
U0933	AB734961	CMC(pH7.3.5%)	⑦	<i>Bacillus clausii</i>	X76440	714/719 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0935	AB734962	CMC(pH7.3.5%)	⑦	<i>Bacillus marisflavi</i>	AF483624	655/659 (99%)	+	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
U0936	AB734963	CMC(pH7.3.5%)	⑧	<i>Streptomyces sogaerensis</i> / <i>Streptomyces rutgersensis</i>	AB184742/AB184170	764/764 (100%)	+	-	-	-	AT	10	ALK	actinobacteria	actinobacteria
U0937	AB734964	CMC(pH7.3.5%)	⑧	<i>Pseudalteromonas tetradonis</i>	AF214730	648/649 (99%), Gaps = 1 / 649 (0%)	+	-	-	-	FA	10	ALK	proteobacteria	gamma
U0938	AB734965	CMC(pH7.3.5%)	⑧	<i>Bacillus marisflavi</i>	AF483624	650/654 (99%), Gaps = 1/654 (0%)	+	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
U0940	AB734966	CMC(pH7.3.5%)	⑧	<i>Bacillus farruginis</i>	AY443036	535/547 (97%), Gaps = 1/547 (0%)	-	-	-	-	AT	3.5	ALK	firmicutes	family Bacillaceae 1
U0941	AB734967	CMC(pH7.3.5%)	⑨	<i>Psychrobacter celer</i>	AY842259	786/790 (99%)	-	-	-	-	AT	15	NE	proteobacteria	gamma
U0942	AB734968	CMC(pH7.3.5%)	⑩	<i>Pseudomonas libanensis</i>	AF057645	742/745 (99%)	+	-	-	-	AT	15	ALK	proteobacteria	gamma
U0943	AB734969	CMC(pH7.3.5%)	⑩	<i>Bacillus aerophilus</i> / <i>Bacillus altitudinis</i>	AJ831844/AJ831842	765/765 (100%)	-	-	-	+	AT	20	ALK	firmicutes	family Bacillaceae 1
U0945	AB734970	CMC(pH7.3.5%)	⑩	<i>Oceanobacillus profundus</i>	DQ386635	767/768 (99%), Gaps = 1/768 (0%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 2
U0948	AB734971	AL(pH7.3.5%)	①	<i>Bacillus clausii</i>	X76440	773/776 (99%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U0951	AB734972	AL(pH7.3.5%)	①	<i>Bacillus aerophilus</i> / <i>Bacillus altitudinis</i> / <i>Bacillus stratosphericus</i>	AJ831844/AJ831842/AJ831841	804/805 (99%), Gaps = 1	-	-	-	+	AT	10	ALK	firmicutes	family Bacillaceae 1
U0956	AB734973	AL(pH7.3.5%)	③	<i>Bacillus marisflavi</i>	AF483624	613/625 (98%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0970	AB734974	AL(pH7.3.5%)	⑧	<i>Bacillus clausii</i>	X76440	632/636 (99%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U0971	AB734975	AL(pH7.3.5%)	⑩	<i>Bacillus pumilus</i> / <i>Bacillus safensis</i>	AY876289/AF234854	703/704 (99%), Gaps = 1/704 (0%)	-	-	-	+	AT	10	ALK	firmicutes	family Bacillaceae 1
U0972	AB734976	AL(pH7.3.5%)	⑩	<i>Bacillus marisflavi</i>	AF483624	817/818 (99%), Gaps = 1/818 (0%)	-	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U0973	AB734977	AL(pH7.3.5%)	⑩	<i>Oceanobacillus picturae</i>	AJ315060	804/805 (99%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U0976	AB734978	XL(pH7.3.5%)	①	<i>Bacillus aquimaris</i>	AF483625	657/666 (98%)	+	+	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
U0977	AB734979	XL(pH7.3.5%)	①	<i>Bacillus gibsonii</i>	X76440	780/782 (99%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U0980	AB734980	XL(pH7.3.5%)	①	<i>Bacillus licheniformis</i>	CP000002	752/754 (99%)	+	-	-	+	AT	15	ALK	firmicutes	family Bacillaceae 1
U0991	AB734981	XL(pH7.3.5%)	③	<i>Bacillus aryabhatai</i>	EF114313	766/766 (100%)	+	-	-	-	FA	15	NE	firmicutes	family Bacillaceae 1
U0992	AB734982	XL(pH7.3.5%)	③	<i>Bacillus altitudinis</i> / <i>Bacillus stratosphericus</i>	AJ831842/AJ831841	827/827 (100%), 827/827 (100%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U1003	AB734983	XL(pH7.3.5%)	⑤	<i>Bacillus stratosphericus</i> / <i>Bacillus aerophilus</i> / <i>Bacillus altitudinis</i>	AJ831841/AJ831844/AJ831842	826/828 (99%), Gaps = 2/828 (0%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U1004	AB734984	XL(pH7.3.5%)	⑤	<i>Bacillus aryabhatai</i>	EF114313	669/670 (99%)	+	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
U1005	AB734985	XL(pH7.3.5%)	⑤	<i>Virgibacillus halodentificans</i>	AY543169	846/851 (99%), Gaps = 1/851 (0%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U1014	AB734986	XL(pH7.3.5%)	⑦	<i>Virgibacillus halodentificans</i>	AY543169	778/780 (99%)	-	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 2
U1017	AB734987	XL(pH7.3.5%)	⑦	<i>Lysinibacillus fusiformis</i>	AJ310083	806/809 (99%)	-	-	-	-	AT	3.5	ALK	firmicutes	family Planococcaceae
U1019	AB734988	XL(pH7.3.5%)	⑦	<i>Bacillus subtilis</i>	AJ276351	819/819 (100%)	+	-	-	+	FA	10	NE	firmicutes	family Bacillaceae 1
U1020	AB734989	XL(pH7.3.5%)	⑧	<i>Bacillus hummeris</i>	HM054773	817/818 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U1021	AB734990	XL(pH7.3.5%)	⑧	<i>Pseudovibrio japonicus</i>	AB246748	759/760 (99%), Gaps = 1/760 (0%)	-	-	-	-	FA	15	ALK	proteobacteria	alpha
U1022	AB734991	XL(pH7.3.5%)	⑧	<i>Virgibacillus halodentificans</i>	AY543169	673/677 (100%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U1023	AB734992	XL(pH7.3.5%)	⑧	<i>Lysinibacillus fusiformis</i>	AJ310083	825/829 (99%), Gaps = 1/829 (0%)	-	-	-	+	FA	10	ALK	firmicutes	family Planococcaceae
U1024	AB734993	XL(pH7.3.5%)	⑧	<i>Bacillus aryabhatai</i>	EF114313	672/672 (100%)	+	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
U1025	AB734994	XL(pH7.3.5%)	⑧	<i>Bacillus marisflavi</i>	AF483624	663/669 (99%), Gaps = 1/669 (0%)	+	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 1
U1026	AB734995	XL(pH7.3.5%)	⑧	<i>Bacillus marisflavi</i>	AF483624	577/587 (98%)	+	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 1
U1027	AB734996	XL(pH7.3.5%)	⑨	<i>Psychrobacter nivalis</i>	AJ313425	796/796 (100%)	-	-	-	-	AT	10	NE	proteobacteria	gamma
U1030	AB734997	XL(pH7.3.5%)	⑩	<i>Bacillus clausii</i>	X76440	761/767 (99%), Gaps = 1/767 (0%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U1032	AB734998	XL(pH7.3.5%)	⑩	<i>Bacillus clausii</i>	X76440	731/735 (99%)	-	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U1035	AB734999	SlpH7.3.5%	①	<i>Bacillus amyloliquefaciens</i>	AB255669	789/789 (100%)	+	-	-	+	FA	10	NE	firmicutes	family Bacillaceae 1
U1037	AB735000	SlpH7.3.5%	①	<i>Bacillus gibsonii</i>	X76440	779/781 (99%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U1041	AB735001	SlpH7.3.5%	①	<i>Bacillus licheniformis</i>	CP000002	579/581 (99%)	+	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 1
U1049	AB735002	SlpH7.3.5%	③	<i>Bacillus pumilus</i>	AY876289	814/818 (99%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U1050	AB735003	SlpH7.3.5%	③	<i>Bacillus licheniformis</i>	CP000002	815/819 (99%)	+	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 1
U1051	AB735004	SlpH7.3.5%	③	<i>Bacillus amyloliquefaciens</i>	AB255669	797/799 (99%)	+	-	-	+	FA	15	NE	firmicutes	family Bacillaceae 1
U1058	AB735005	SlpH7.3.5%	⑤	<i>Bacillus aerophilus</i> / <i>Bacillus altitudinis</i>	AJ831844/AJ831842	766/766 (100%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U1061	AB735006	SlpH7.3.5%	⑤	<i>Bacillus clausii</i>	X76440	773/776 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U1063	AB735007	SlpH7.3.5%	⑥	<i>Bacillus pumilus</i> / <i>Bacillus safensis</i>	AY876289/AF234854	801/807 (99%)	-	-	-	+	AT	15	ALK	firmicutes	family Bacillaceae 1

Supplementary Table 2

Isolate no.	Accession number of isolates	Isolation medium	Specimen Number	species / tentative species	Accession number of species / tentative species	Identities	Degrading activities on polysaccharides				Requirement of oxygen	Maximum NaCl concentration for growth	pH tolerance	phylum	family
							S	CMC	AI	XL					
U1064	AB735008	S(pH7.3.5%)	⑥	<i>Bacillus methylotrophicus</i>	EU194897	825/827 (99%), Gaps = 1/827 (0%)	+	-	-	+	AT	10	NE	firmicutes	family Bacillaceae 1
U1066	AB735009	S(pH7.3.5%)	⑥	<i>Bacillus subtilis</i>	AJ276351	819/819 (100%)	+	-	-	+	AT	10	NE	firmicutes	family Bacillaceae 1
U1068	AB735010	S(pH7.3.5%)	⑥	<i>Bacillus licheniformis</i>	CP000002	766/767 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1069	AB735011	S(pH7.3.5%)	⑥	<i>Bacillus licheniformis</i>	CP000002	665/668 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1070	AB735012	S(pH7.3.5%)	⑦	<i>Bacillus licheniformis</i>	CP000002	759/760 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1072	AB735013	S(pH7.3.5%)	⑦	<i>Bacillus hananensis</i>	HM054473	810/811 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U1074	AB735014	S(pH7.3.5%)	⑦	<i>Bacillus aryabhattai</i>	EF114313	733/733 (100%)	+	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
U1075	AB735015	S(pH7.3.5%)	⑧	<i>Bacillus licheniformis</i>	CP000002	781/783 (99%)	+	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 1
U1077	AB735016	S(pH7.3.5%)	⑧	<i>Bacillus stratosphericus</i>	AJ831841	761/762 (99%), Gaps = 1/762 (0%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U1078	AB735017	S(pH7.3.5%)	⑧	<i>Bacillus marisflavi</i>	AF483624	713/717 (99%)	-	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1079	AB735018	S(pH7.3.5%)	⑧	<i>Bacillus cereus</i>	AE016877	813/813 (100%)	+	+	-	-	FA	3.5	NE	firmicutes	family Bacillaceae 1
U1080	AB735019	S(pH7.3.5%)	⑧	<i>Bacillus aryabhattai</i>	EF114313	828/829 (99%), Gaps = 1/829 (0%)	+	-	-	-	A	10	NE	firmicutes	family Bacillaceae 1
U1081	AB735020	S(pH7.3.5%)	⑧	<i>Bacillus amyloliquefaciens</i>	AB255669	761/763 (99%)	+	+	-	-	FA	10	NE	firmicutes	family Bacillaceae 1
U1082	AB735021	S(pH7.3.5%)	⑧	<i>Bacillus licheniformis</i>	CP000002	784/786 (99%), Gaps = 1/786 (0%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1083	AB735022	S(pH7.3.5%)	⑧	<i>Bacillus clausii</i>	X76440	773/776 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1084	AB735023	S(pH7.3.5%)	⑧	<i>Bacillus marisflavi</i>	AF483624	663/673 (98%)	-	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1086	AB735024	S(pH7.3.5%)	⑧	<i>Bacillus altitudinis/Bacillus stratosphericus</i>	AJ831842/AJ831841	817/818 (99%), Gaps = 1	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U1088	AB735025	LB(pH7.3.5%)	①	<i>Bacillus clausii</i>	X76440	666/668 (99%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U1089	AB735026	LB(pH7.3.5%)	①	<i>Virgibacillus halodentificans</i>	AY543169	675/675 (100%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
U1099	AB735027	LB(pH7.3.5%)	③	<i>Bacillus aerophilus/Bacillus altitudinis/Bacillus stratosphericus</i>	AJ831844/AJ831842/AJ831841	696/698 (99%)	-	-	-	+	FA	10	ALK	firmicutes	family Bacillaceae 1
U1100	AB735028	LB(pH7.3.5%)	③	<i>Nocardopsis lucentensis</i>	X97888	545/549 (99%)	-	-	-	-	AT	10	ALK	actinobacteria	actinobacteria
U1102	AB735029	LB(pH7.3.5%)	③	<i>Bacillus clausii</i>	X76440	574/578 (99%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 1
U1109	AB735030	LB(pH7.3.5%)	⑤	<i>Bacillus licheniformis</i>	CP000002	733/735 (99%)	-	-	-	+	FA	20	ALK	firmicutes	family Bacillaceae 1
U1112	AB735031	LB(pH7.3.5%)	⑥	<i>Bacillus clausii</i>	X76440	774/776 (99%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U1113	AB735032	LB(pH7.3.5%)	⑥	<i>Bacillus pumilus/Bacillus safensis</i>	AY876289/AF234854	821/821 (100%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U1115	AB735033	LB(pH7.3.5%)	⑥	<i>Virgibacillus halodentificans</i>	AY543169	810/810 (100%)	-	-	-	-	FA	25	ALK	firmicutes	family Bacillaceae 2
U1116	AB735034	LB(pH7.3.5%)	⑥	<i>Bacillus marisflavi</i>	AF483624	813/818 (99%)	-	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1117	AB735035	LB(pH7.3.5%)	⑥	<i>Bacillus altitudinis/Bacillus stratosphericus</i>	AJ831842/AJ831841	695/695 (100%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U1118	AB735036	LB(pH7.3.5%)	⑦	<i>Bacillus altitudinis/Bacillus stratosphericus</i>	AJ831842/AJ831841	789/789 (100%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
U1119	AB735037	LB(pH7.3.5%)	⑦	<i>Ferrimonas senticii</i>	DQ278094	715/736 (97%), Gaps = 2/736 (0%)	+	-	-	-	FA	3.5	ALK	proteobacteria	gamma
U1120	AB735038	LB(pH7.3.5%)	⑦	<i>Oceanobacillus chironomi</i>	DQ298074	712/740 (96%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 2
U1121	AB735039	LB(pH7.3.5%)	⑦	<i>Bacillus hananensis</i>	HM054473	817/818 (99%)	+	-	-	-	A	15	ALK	firmicutes	family Bacillaceae 1
U1123	AB735040	LB(pH7.3.5%)	⑦	<i>Lysinibacillus fusiformis</i>	AJ310083	778/781 (99%)	-	-	-	-	FA	3.5	ALK	firmicutes	family Planococcaceae
U1124	AB735041	LB(pH7.3.5%)	⑧	<i>Bacillus clausii</i>	X76440	733/734 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
U1125	AB735042	LB(pH7.3.5%)	⑧	<i>Bacillus altitudinis/Bacillus stratosphericus</i>	AJ831842/AJ831841	694/694 (100%)	-	-	-	+	FA	10	ALK	firmicutes	family Bacillaceae 1
U1126	AB735043	LB(pH7.3.5%)	⑧	<i>Bacillus clausii</i>	X76440	680/682 (99%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
U1127	AB735044	LB(pH7.3.5%)	⑧	<i>Virgibacillus dolodemensis</i>	AY822043	778/779 (99%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
U1128	AB735045	LB(pH7.3.5%)	⑨	<i>Williamsia serinedens</i>	AM283464	752/760 (98%)	-	-	-	-	FA	3.5	NE	actinobacteria	actinobacteria
U1129	AB735046	LB(pH7.3.5%)	⑩	<i>Bacillus hananensis</i>	HM054473	816/818 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
U1133	AB735047	LB(pH7.3.5%)	⑩	<i>Bacillus clausii</i>	X76440	763/767 (99%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1

*: ①-⑩ indicated samples from black adult ①, green adult ③, black small ⑤, green small ⑥, black juvenile ⑦, and green juvenile ⑧ sea cucumbers, respectively. ⑨ and ⑩ indicated samples from seawater ⑨ and sea sediment ⑩, respectively. Abbreviations: S, starch; CMC, carboxymethyl cellulose sodium salt; AI, alginate; XL, xylan; FA, facultative anaerobic bacteria; AT, anaerobic tolerant bacteria; A, aerobic bacteria; NE, neutrophilic bacteria; ALK, alkaliphilic bacteria. Display of more than one species in the column of species indicated the same identity in the comparison range.