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Diversity and function of aerobic culturable bacteria in the intestine of deep-sea holothurian

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Abstract

Ninety-two aerobic culturable bacterial strains were isolated from each part of the intestine of the deep-sea holothurian collected at 32°30′N, 129°09′E (southeast of Fukue Island, Nagasaki, Japan) and water depth of 236 m in November 2010. The temperature of seawater at the water depth of the sampling point was estimated to be ca.13-14 °C from data in Japan Meteorological Agency. By partial 16S rRNA gene sequences of the isolates, the isolates belonged to 45 nearest type strain species (below, referred to as species). High diversity was observed in the genera *Bacillus* (21 species) and *Vibrio* (6 species). The bacterial diversity was similar among three parts, i.e. anterior, mid and posterior parts of the intestine and 14 species were detected in multiple parts of the intestine. Most isolates showed various polysaccharide degradation activities but few isolates showed alginate or agar degradation activities probably because there were no seaweeds containing alginate or agar in this deep-sea. Comparing the functions and properties of several species in three parts, the posterior part was likely to be different from the anterior or mid parts.

Key words: aerobic culturable bacteria; deep-sea holothurian; diversity; function; 16S rRNA gene

Introduction

Deep-sea is one of unexplored regions even today. Holothurians (~1430 species) are found on various sea floors from deep sea floors to intertidal areas [4,9]. Holothurians belong to phylum Echinodermata and their diet is detritus such as organic matter, microalgae, and bacteria[6,7,11].

48 Gut bacteria play an important role in digestion of diets. Studies on
49 bacteria associated with Holothurians were reported only for *Holothuria*
50 *atra*[10] and *Molpadia musculus*[1,2]. Ward-Rainey et al. reported partial
51 aerobic bacterial flora of *Holothuria atra*. [10] In their report, only 23
52 isolates were characterized by 16S rRNA gene sequences analysis (the
53 first 300 nucleotides) and they were affiliated to the genera *Vibrio* and
54 *Bacillus*. On the other hands, Amaro et al. used non-culturing methods to
55 analyze bacterial community of abyssal holothurian, *Molpadia*
56 *musculus*.[2] Their results suggested that the gut bacterial composition
57 was similar to that of the organic matter-rich sediments. Members of
58 Cytophaga- Flavobacteria-Bacteroides (CFB) group dominated in the
59 bacterial community[2]. Recently, they also found that ca. 82% of total
60 bacterial OTUs (Operational Taxonomic Unit) were common between the
61 gut contents and the surrounding sediments[1]. Enomoto et al. also
62 reported recently that γ-*Proteobacteria* members were mainly isolated as
63 culturable bacteria from the intestine of *Apostichopus japonicus*[3]. Using
64 the molecular techniques, they also found that *Proteobacteria* members
65 were main metabolically active microbial populations in the intestine of
66 *Apostichopus japonicus*.
67 Gut microorganisms play an important role in digestion of diets,
68 but the diversity and function of aerobic culturable bacteria in the
69 intestine of the deep-sea holothurian are still unclear.
70 In this report, we isolated ninety-two aerobic culturable bacterial
71 strains from each part of the intestine of the deep-sea holothurian
72 collected at the southeast of Fukue Island, Nagasaki, Japan,
73 water depth of 236 m and in November 2010. We found that the aerobic
74 culturable isolates belonged to 45 nearest type strain species (below,
75 referred to as species). The bacterial diversity was similar among three

parts, i.e. anterior, mid and posterior parts of the intestine. Most isolates showed various polysaccharide degradation activities but few isolates showed alginate or agar degradation activities probably because there were no seaweeds in deep-sea. On the other hand, when we compared the functions and properties of several species in three parts, the posterior part was likely to be different from the anterior or mid parts. Maybe the posterior part was related to the digestion of polysaccharides or high salt environment.

Materials and Methods

Sample collection and dissection. Deep-sea holothurian specimen was collected at southeast of Fukue Island, Nagasaki, Japan (32°30′N, 129°09′E), at a water depth of 236 m in November 21, 2010 (Fig.1). The temperature of seawater at the water depth of the sampling point was estimated to be ca.13-14 °C from data in Japan Meteorological Agency. The specimen was kept in icebox and aseptically dissected in our laboratory in November 24, 2010. Whole intestine was excised from the animal body aseptically using sterilized instruments. Fraction of intestine was carried out according to Shimizu et al. [8] The intact intestine was divided into three parts, the anterior part (0.71g), the mid part (0.88g) and the posterior part (1.80g) (Fig. 2). To isolate bacteria from both intestinal wall and contents, 1 ml of saline was added to each part and each part was crushed and mixed enough. Each suspension thus obtained was used for isolation of bacteria and 50 μ l of the each suspension was spread on plates.

104

105 *Growth media*. Luria-Bertani medium (LB) and Horikoshi medium
106 were used basically. But NaCl concentration was 3.5% instead of 1%.
107 Polysaccharides such as carboxymethyl cellulose sodium salt (CMC)
108 (Wako pure chemicals, Osaka, Japan), xylan (Sigma), sodium alginate
109 (Wako pure chemicals, Osaka, Japan) and soluble starch (nacalai tesque,
110 Kyoto, Japan) were added to Horikoshi medium as carbon sources (final
111 concentration 1%).

112 LB solid medium (pH 7) contained 1% tryptone (Difco), 0.5%
113 yeast extract (Difco), 3.5% NaCl, and 1.5 % agar (Wako pure chemicals,
114 Osaka, Japan). Horikoshi solid medium (pH 7) contained 1%
115 polysaccharide, 0.5% peptone (BD), 0.5% yeast extract, 0.1% KH_2PO_4,
116 0.02% $MgSO_4 \cdot 7H_2O$, 3.5% NaCl, and 2% agar. Sodium alginate solid
117 medium contained 2.5% agar. For 10% NaCl media, NaCl concentration
118 of growth media was 10% instead of 3.5%.

119 For alkaline agar plates, Na_2CO_3 (autoclaved separately) was
120 added to neutral agar medium (final pH: pH10.3-10.5). Na_2CO_3
121 concentration of alkaline plate was 1%.

122

123 *Isolation of bacteria*. Fifty µl of the gut suspensions was directly
124 plated on agar plates without enrichment culture. High salt concentration
125 or high pH were used for isolation conditions to isolate various bacteria
126 because marine water is semi-alkaline pH and contains 3.5% NaCl.
127 Seventeen different media were prepared by combination of pH, NaCl
128 concentration and carbon source (Table 1). The plates were incubated at
129 30 °C aerobically for two weeks to obtain slowly growing bacteria.
130 Bacteria were isolated from each plate, purified and stored in slants for
131 further analysis.

Physiological and biochemical characteristics of isolates.

Polysaccharide degradation activities were detected by plate methods using CMC, xylan, alginate, starch 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% $MgCl_2 \cdot 6H_2O$, 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% $MgSO_4$, 0.5% $MgCl_2$, 0.1% $CaSO_4$, 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 alginate lyase.

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, Na_2CO_3 (autoclaved separately) was added to

160 neutral agar medium (final pH: pH10.3-10.5). Na_2CO_3 concentration of
161 alkaline plate was 1%.
162
163 All isolates were tested for salt tolerance: 0%, 3.5%, 10%, 15%,
164 20%, 25% NaCl (w/v), pH tolerance (pH7 and pH10) and effect of
165 oxygen.
166 Anaerobic growth was examined using gaspak (COSMO BIO) at
167 30 °C for two weeks, and then growth condition was changed to the
168 aerobic condition at 30°C for two weeks. The isolates were assigned to
169 three groups, facultative anaerobic bacteria (FA), anaerobic tolerant
170 bacteria (AT) and aerobic bacteria (A). Facultative anaerobic bacteria
171 form colony in both aerobic and anaerobic cultivation. Anaerobic
172 tolerant bacteria do not form colony in anaerobic condition for two
173 weeks but form colony in aerobic cultivation after the anaerobic
174 cultivation. Aerobic bacteria do not form colony in anaerobic condition
175 for two weeks and also do not form colony in aerobic cultivation after
176 the anaerobic cultivation. Growth ability at various conditions of
177 salinity or pH was measured at 30 °C for two weeks. The isolates were
178 divided into two groups by effect of pH on growth, neutrophilic bacteria
179 (NE) that grew only at pH7, and alkaliphilic/alkali-tolerant bacteria
180 (ALK) that grew both at pH 7 and pH10.
181
182 *Molecular identification of the isolates.* Partial analysis of 16S
183 ribosomal RNA (rRNA) gene of the isolates was carried out. The 16S
184 rRNA gene was amplified using bacterial primers 27f
185 (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTT
186 GTTACGACTT-3') and the purified PCR product was sequenced with
187 dideoxynucleotide chain-termination method using 3130 or 3730 DNA

sequencer (Applied Biosystems). Primers 27F, 520R (5'-ACCGCGGCT GCTGGC-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 AB741781 through AB741873 except AB741857 (Supplementary Table 1; see JJSE 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). Each isolate was assigned to nearest type strain species (Supplementary Table 1; see JJSE Web site). When isolate showed more than 97% identities with some type strain sequences, the isolate was assigned to nearest type strain species (below, referred to as species). When isolate showed less than 97% identities with any type strain sequences, the isolate was assigned to the tentative nearest type strain species (below, referred to as tentative species).

Results

Isolation of bacteria

Fig.2 shows photographs of the deep-sea holothurian and its dissection (C), including anterior intestine (1), mid intestine (2), posterior intestine (3), Polian vesicle (4) and respiratory trees (5). But Cuvierian tubules were not detected in the specimen. The each intestine suspension was directly plated on agar plates and seventeen isolation

media were used. Table 1 summarized number of the isolates obtained by different cultural conditions. The intact intestine was divided into three parts, the anterior part, the mid part and the posterior part. The each part was crushed and mixed enough and the suspensions thus obtained were used for isolation of bacteria. Number of colony forming units (cfu) per g of the anterior, mid and posterior parts were 1.4×10^4 cfu/g, 0.6×10^4 cfu/g and 0.58×10^4 cfu/g in LB medium, respectively. Similar cfu numbers were obtained in Horikoshi media (pH7), but lower cfu numbers in the alkaline Horikoshi media (ca. pH10.3).

Twenty-four, 33 and 35 isolates were obtained from the anterior, mid and posterior suspensions, respectively. In total, 92 isolates were purified and analyzed further.

Phylogenic analysis of bacterial isolates

The partial 16S rRNA gene sequences were done and compared with other sequences in DDBJ database using BLAST program and compared with the type strain sequences in Ribosomal database project (RDP). Table 2 summarized the species/the tentative species of the isolates as determined via BLAST (Supplementary Table 1JJSE Web site). By partial 16S rRNA gene sequences of the isolates, the isolates belonged to 45 species. Fourteen species were detected in multiple locations of three parts, i.e. anterior, mid and posterior parts of the intestine. The isolates belonged to the phyla Firmicutes (33 species) and Proteobacteria (12 species) (Table 2). Among 33 species of the phylum Firmicutes, 21 species belonged to the family Bacillaceae 1, the genus *Bacillus*. Ten species belonged to the family Bacillaceae 2, the genera *Gracilibacillus*, *Halobacillus*, *Oceanobacillus*, *Thalassobacillus* and *Virgibacillus*. Twelve species of the phylum Proteobacteria belonged

to the genera *Vibrio, Halomonas, Photobacterium, Pseudomonas* and *Marinobacter.* Among them, high diversity was found in the genera *Bacillus* and *Vibrio*. The closest relatives of these isolates were observed in various sea environments. The bacterial diversity was similar among three parts, i.e. anterior, mid and posterior parts of the intestine (Fig.3) and 14 species (indicated by star in Table 2) were detected in multiple parts of the intestine. But, the number of species belonged to the family Bacillaceae 2 decreased in the posterior part of the intestine compared with those in the anterior or mid parts of the intestine (Fig.3).

Three isolates (isolate no. C214, C254 and C271) showed less than 97% identities with any type strain sequences. The isolates were assigned to the tentative species. (Supplementary Table 1; see JJSE Web site).

Polysaccharide degradation ability of isolates

Many isolates from the specimen showed polysaccharides degradation ability and degraded one or more substrates (S, CMC, AL and XL). Twenty-eight, 12, 3 and 12 species showed amylase activity, cellulase activity, alginate lyase activity and xylanase activity, respectively (Fig.4, Supplementary Table 1; see JJSE Web site). No agarase activity was observed in all isolates.

Amylase producing isolates were mainly affiliated with the genus *Bacillus* (15 species). Twelve species such as *Bacillus horikoshii, B. hunanensis, B. licheniformis, B. megaterium, B. vietnamensis, Halobacillus kuroshimensis, H. trueperi, Photobacterium rosenbergii, Pseudomonas cedrina, P. libanensis, Vibrio pomeroyi,* and *V. rotiferianus* were found in multiple locations of three parts, i.e. anterior, mid and posterior parts of the intestine.

Most cellulase positive isolates belonged to the genera *Bacillus* (5 species) and *Vibrio* (4species). Three species such as *Jeotgalibacillus campisalis, Vibrio pomeroyi* and *V. rotiferianus* were found in multiple locations. Only 3 isolates producing alginate-lyase were affiliated with *Gracilibacillus dipsosauri, Pseudomonas synxantha* and *Vibrio agarivorans.* All isolates producing xylanase were most closely related to the genera *Bacillus* (9 species) and *Pseudomonas* (3 species). One species, *Pseudomonas libanensis* was found in multiple locations.
Several species showed multiple polysaccharides degradation activities. On the other hand, 11 species had no polysaccharides degradation ability and 7 species of them in the anterior or mid parts of the intestine belonged to the family Bacillaceae 2 but the number of them decreased in the posterior part of the intestine (Fig.4). It was observed that in the posterior part, the number of xylan degrading species related to the family Bacilliaceae 1 increased and the number of starch degrading species related to the order γ-Proteobacteria also increased (Fig.4).

Physiological characteristics of the isolates

Fig.5 and Supplementary Table 1 (JJSE Web site) showed effect of anaerobic condition for growth of the isolates. The isolates were divided into three groups, facultative anaerobic bacteria (FA), anaerobic tolerant bacteria (AT) and aerobic bacteria (A). Twenty-five FA species were mainly affiliated with the phylum Proteobacteria and the family Bacillaceae 1. Six species such as *Bacillus licheniformis, Photobacterium rosenbergii, Pseudomonas cedrina, P. libanensis, Vibrio pomeroyi* and *V. rotiferianus* were found in multiple locations. Thirty AT species were mainly affiliated to the genera *Bacillus* (16 species), *Gracilibacillus, Halobacillus, Oceanobacillus* and *Virgibacillus.* Only

300 one aerobic (A) species, *Halobacillus kuroshimensis* was found in
301 multiple locations. The species belonging to the family Bacillaceae 2
302 were mainly affiliated to AT group and species belonging to the family
303 Bacillaceae 1 were mainly affiliated to FA or AT groups (Fig.5). The
304 species belonging to the phylum Proteobacteria were mainly affiliated to
305 FA group (Fig.5).
306 High salt concentration or high pH were used for isolation
307 conditions to isolate various bacteria because marine water is
308 semi-alkaline pH and contains 3.5% NaCl. Salinity tolerance of the
309 isolates was examined (Fig.6, and Supplementary Table 1; JJSE Web
310 site). The isolates most closely related to *Halobacillus kuroshimensis*
311 and *Bacillus clausii* showed the highest salt tolerance (25 % NaCl) and
312 some strains were able to grow in absent of NaCl. Twelve species were
313 halophilic (20-25% NaCl conc.) and mainly belonged to the family
314 Bacillaceae 2, the genera*Halobacillus, Virgibacillus* and *Oceanobacillus*.
315 Thirty-two species were moderate halophilic (10- and18 species
316 belonged to the family Bacillaceae 1, the genus *Bacillus* and 9 species
317 belonged to γ-proteobacteria such as the genera *Pseudomonas, Vibrio*
318 and *Photobacterium*.Seven species were slight halophiles (3.5% NaCl)
319 belonged to the genera *Vibrio*, *Photobacterium, Jeotgalibacillus Bacillus*.
320 It appears that the species belonging to the family Bacillaceae 2 are most
321 salt-tolerant (more than 20% NaCl) and those belonging to the phylum
322 Proteobacteria or the family Bacillaceae 1 were moderate halophilic
323 (10-)(Fig.6). It was observed that number of species related to slight
324 halophiles (3.5%) decreased in the posterior part compared with those in
325 other parts.
326 All isolates were examined for growth responses to pH shift
327 (pH7→pH10 or pH10→pH7. All alkaliphilic/alkali-tolerant strains

isolated from alkali medium were able to grow at pH 7, while half of species isolated from pH 7 were able to grow at pH 10. Nineteen species were the neutrophilic species (NE) growing only at pH7. Eleven species were affiliated with the genus *Bacillus*.

Discussion

In this report, we isolated aerobic culturable bacteria from each part of the gut of deep-sea holothurian using different culture conditions. The deep-sea holothurian was collected at southeast of Fukue Island, Nagasaki, Japan (32°30′N, 129°09′E), at a water depth of 236 m in November 21, 2010. Ninety-two aerobic culturable bacterial strains were isolated from each part of the intestine of deep-sea holothurian. By partial 16S rRNA gene sequences of the isolates, the isolates belonged to 45 species. The bacterial diversity was similar among three parts, i.e. anterior, mid and posterior parts of the intestine and 14 species were detected in multiple parts of the intestine. But, the number of species belonged to the family Bacillaceae 2 decreased in the posterior part of the intestine compared with those in the anterior or mid parts of the intestine. (We will discuss this later.)

As shown in Table2, the isolates belonged to the phyla Firmicutes (33 species) and Proteobacteria (12 species). Among 33 species of the phylum Firmicutes, 21 species belonged to the family Bacillaceae 1, the genus *Bacillus*. Recently, Enomoto et al. reported that Proteobacteria members were mainly isolated as culturable bacteria from the intestine of *Apostichopus japonicus*[3]. These results suggested that the sea environments such as deep sea or intertidal areas maybe affected diversity of aerobic culturable bacteria.

Detritus is organic materials and is used as a source of nutrient for

356 detritus feeders[5]. Most important components of detritus are recalcitrant
357 polysaccharides and bacteria are the main decomposers that degrade
358 these materials. Therefore, we analyzed polysaccharide degradation of
359 the isolates. We found that many isolates showed various polysaccharide
360 degradation activities. High diversity was observed in starch degradation
361 isolates, suggesting the large amount storage of starch in detritus, for
362 example algae. But, there were few isolates showing alginate or agar
363 degradation activities probably because deep-sea was not suitable area
364 for seaweeds which contained a lot of alginate or agar. As mentioned in
365 Fig.4, 11 species had no polysaccharides degradation ability and 7
366 species of them in the anterior or mid parts of the intestine belonged to
367 the family Bacillaceae 2 but the number of them decreased in the
368 posterior part of the intestine. It was observed that in the posterior part,
369 the number of xylan degrading species related to the family Bacilliaceae
370 1 increased and the number of starch degrading species related to the
371 order γ-Proteobacteria also increased (Fig.4). It was also observed that
372 number of species related to slight halophiles (3.5%) decreased in the
373 posterior part compared with those in other parts. These results
374 suggested that the posterior part had a different role or environment
375 compared with the anterior or mid parts, maybe the posterior part was
376 involved in the digestion of polysaccharides.
377 We found that almost all isolates were facultative anaerobic
378 bacteria or anaerobic tolerant bacteria. It appears that oxygen will enter
379 the intestine of the sea cucumber from the mouth with the detritus food
380 and also some amount can penetrate from the body tissues. These results
381 suggested that the aerobic culturable isolates potentially contributed to
382 digest detritus and supply metabolic products (minor components and
383 vitamins) to their host sea cucumber.

384 Three isolates (isolate no. C214, C254 and C271) showed less than
385 96% identities with any type strain sequences and two of them were
386 obtained from alkaline plates and 10% NaCl. These results suggested
387 that the intestines of deep-sea holothurians were still new resource for
388 new species.
389 The temperature of seawater at the water depth of the sampling
390 point was estimated to be ca.13-14 °C in November 2010 from data of
391 Japan Meteorological Agency. But, it was surprising that the full year
392 temperature of the sampling point was estimated to be ca.12-15 °C
393 throughout the year (from data in Japan Meteorological Agency). These
394 results suggested that this deep-sea environment seemed a little low
395 temperature but more suitable environment for deep-sea holothurian and
396 their intestinal bacteria.
397 In this study, only one specimen, deep-sea holothurian was obtained
398 and investigated. Therefore, future challenges remain regarding
399 individual variations, morphological descriptions, haplotypes of host
400 species and surrounding environments including sediments.
401
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403
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Figure legends

Fig.1 Map of sampling site (arrow) and environmental conditions.
Sampling point : 32°30′N, 129°09′E (Southeast of Fukue Island, Nagasaki, Japan) Water depth: 236 m, Sampling data: November 21, 2010, Temperature of water depth 200 m : ca.13-14℃, Sea surface temperature: ca. 21-22℃（Data from Japan Meteorological Agency）

Fig. 2 Photographs of the deep-sea holothurian (dorsal side A, ventral side B) and its dissection (C), including anterior intestine (1), mid intestine (2), posterior intestine (3), Polian vesicle (4) and respiratory trees (5). Cuvierian tubules were not detected in the sample.

Fig.3 Number of the species of isolates in each part of the intestine
The species were divided into five groups: the family Bacillaceae 1 (gray box), the family Bacillaceae 2 (vertical stripes), the family Planococcaceae (dotted box), the family Staphylococcaceae (black box) and the order γ-Proteobacteria (diagonal stripes box). Fig.3 was summarized from Supplementary Tables 1 (JJSE web site).

Fig.4 Number of the species of isolates degrading various polysaccharides
The species were divided into five groups as shown in Fig.3. S, CMC, AL, XL and None indicated starch degradation activity (S), CMC degradation activity (CMC), alginate degradation activity (AL), xylan degradation activity (XL) and no degradation activity (None), respectively. No isolates showed agarase activity. Fig.4 was summarized from Supplementary Tables 1 (JJSE web site).

Fig.5 Number of the species of isolates classified by effect of oxygen on the growth

The species were divided into five groups as shown in Fig.3. 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. Fig.5 was summarized from Supplementary Tables 1 (JJSE web site).

Fig.6 Number of the species of isolates classified by effect of NaCl concentration on the growth

The species were divided into five groups as shown in Fig.3. Fig.6 was summarized from Supplementary Tables 1 (JJSE web site).

Fig.1 T. Kudo

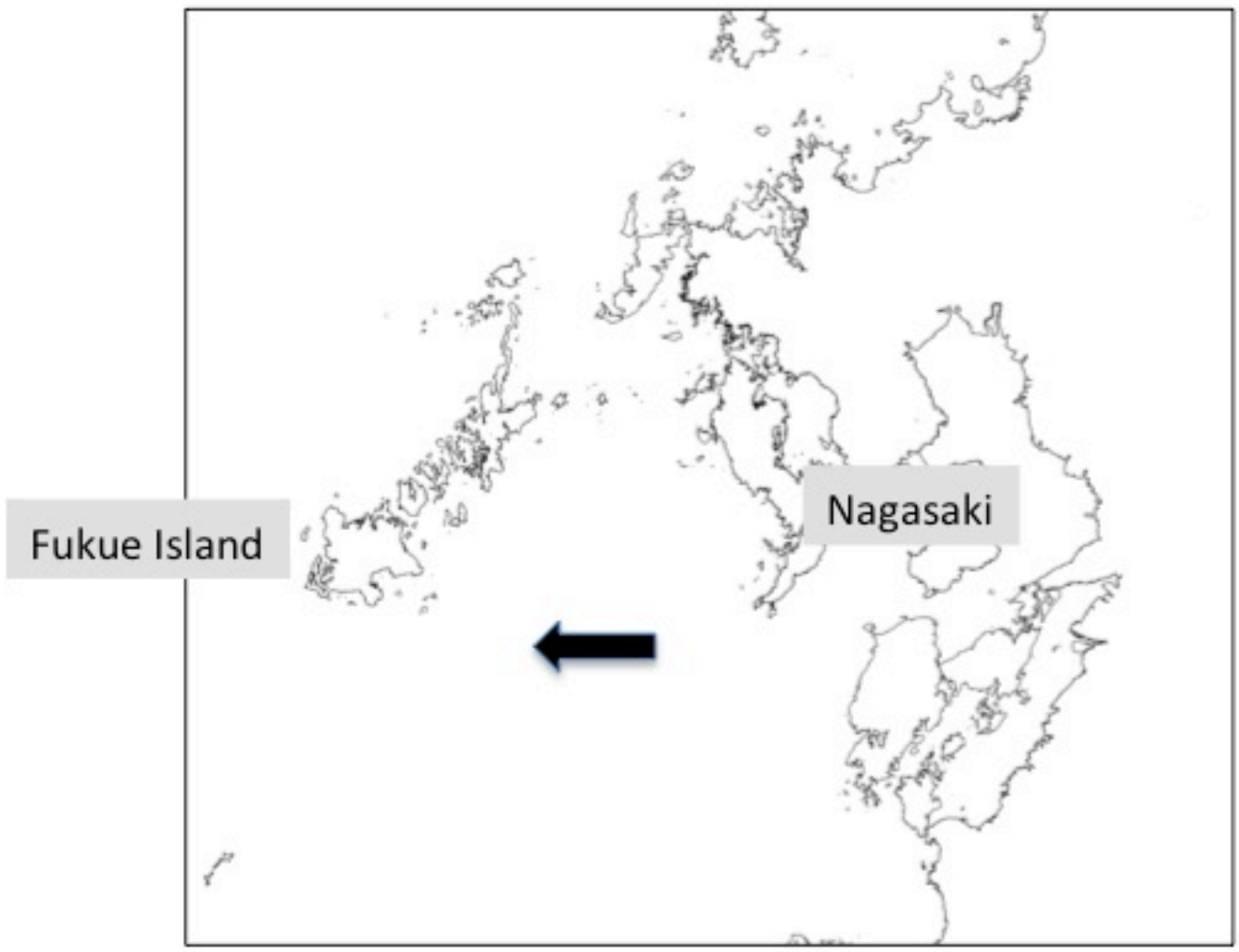

Fig.1 Map of sampling site (arrow) and environmental conditions.
Sampling point : 32°30′N, 129°09′E
(Southeast of Fukue Island, Nagasaki, Japan)
Water depth: 236 m
Sampling data: November 21, 2010

Temperature of water depth 200 m : ca.13-14 ℃
Sea surface temperature: ca. 21-22 ℃
（Data from Japan Meteorological Agency)

Fig.2 T.Kudo

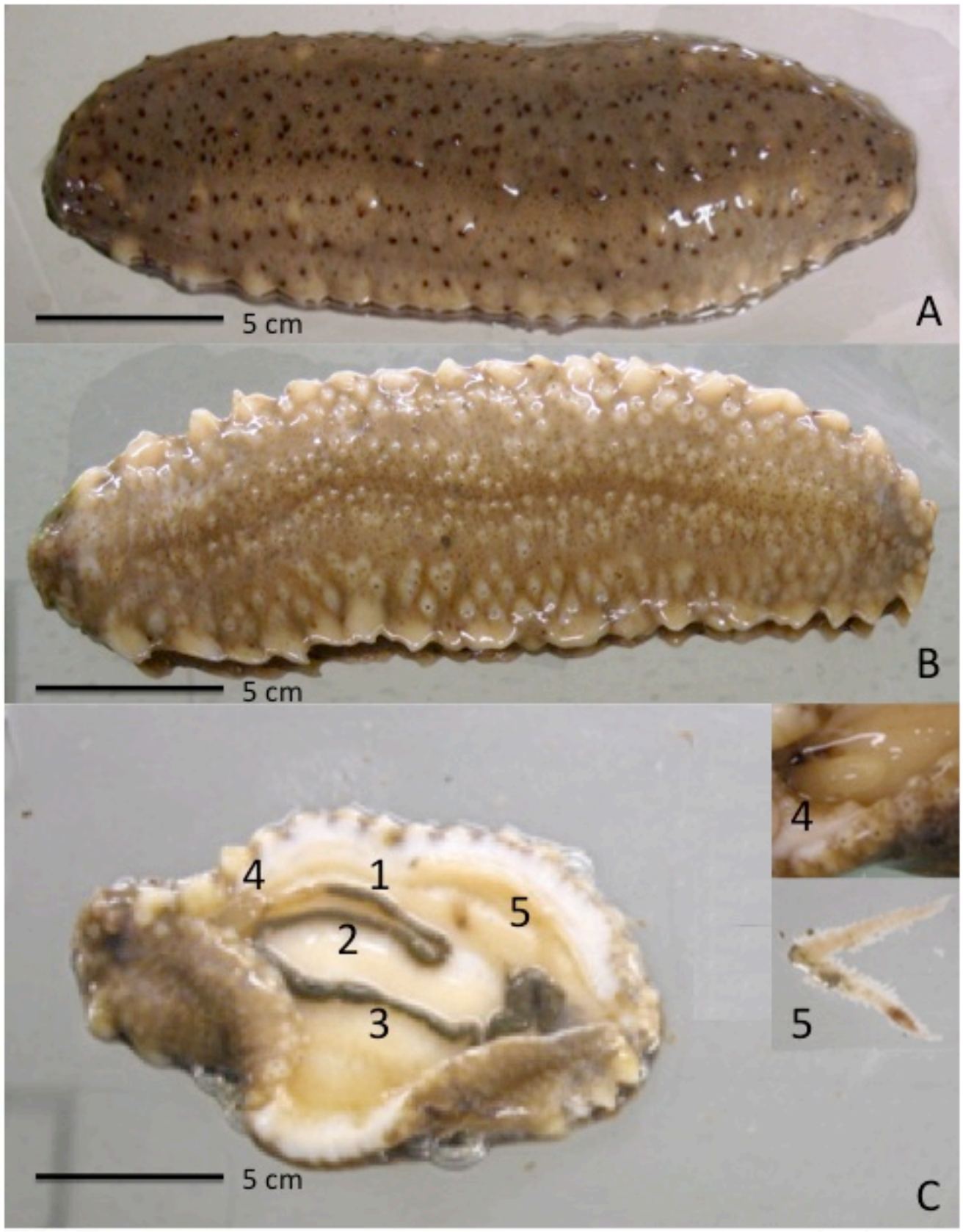

Fig. 2 . Photograph of the deep-sea holothurian (dorsal side A, ventral sideB) and its dissection (C), including anterior intestine (1), mid intestine (2), posterior intestine (3), Polian vesicle (4) and respiratory trees (5). Cuvierian tubules were not detected in the sample.

Fig.3 Kudo

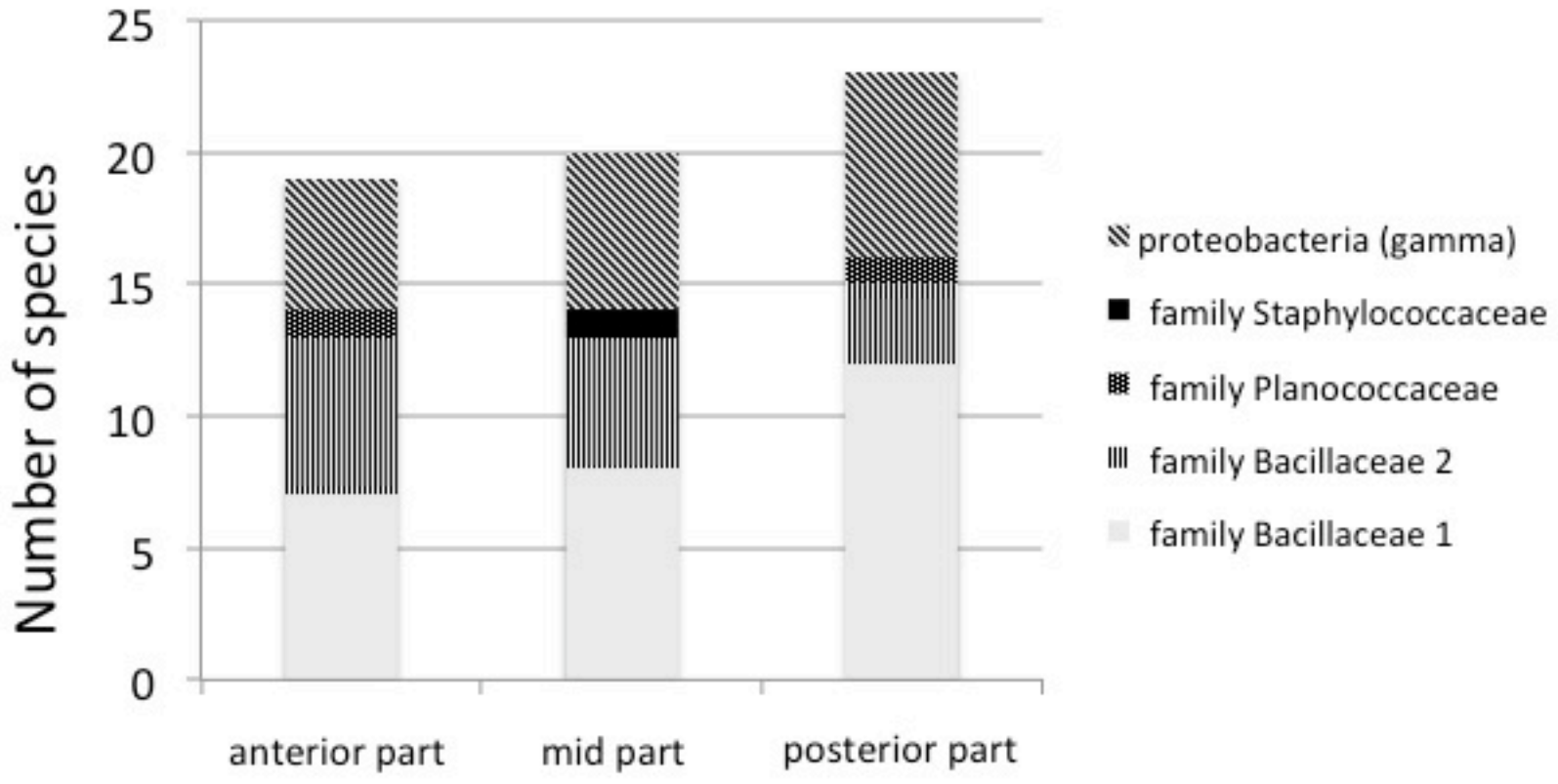

Fig.3 Number of the species of isolates in each part of the intestine The species were divided into five groups: the family Bacillaceae 1 (gray box), the family Bacillaceae 2 (vertical stripes), the family Planococcaceae (dotted box), the family Staphylococcaceae (black box) and the order g-Proteobacteria (diagonal stripes box). Fig.3 was summarized from Supplementary Tables 1 (JJSE web site).

Fig.4 kudo

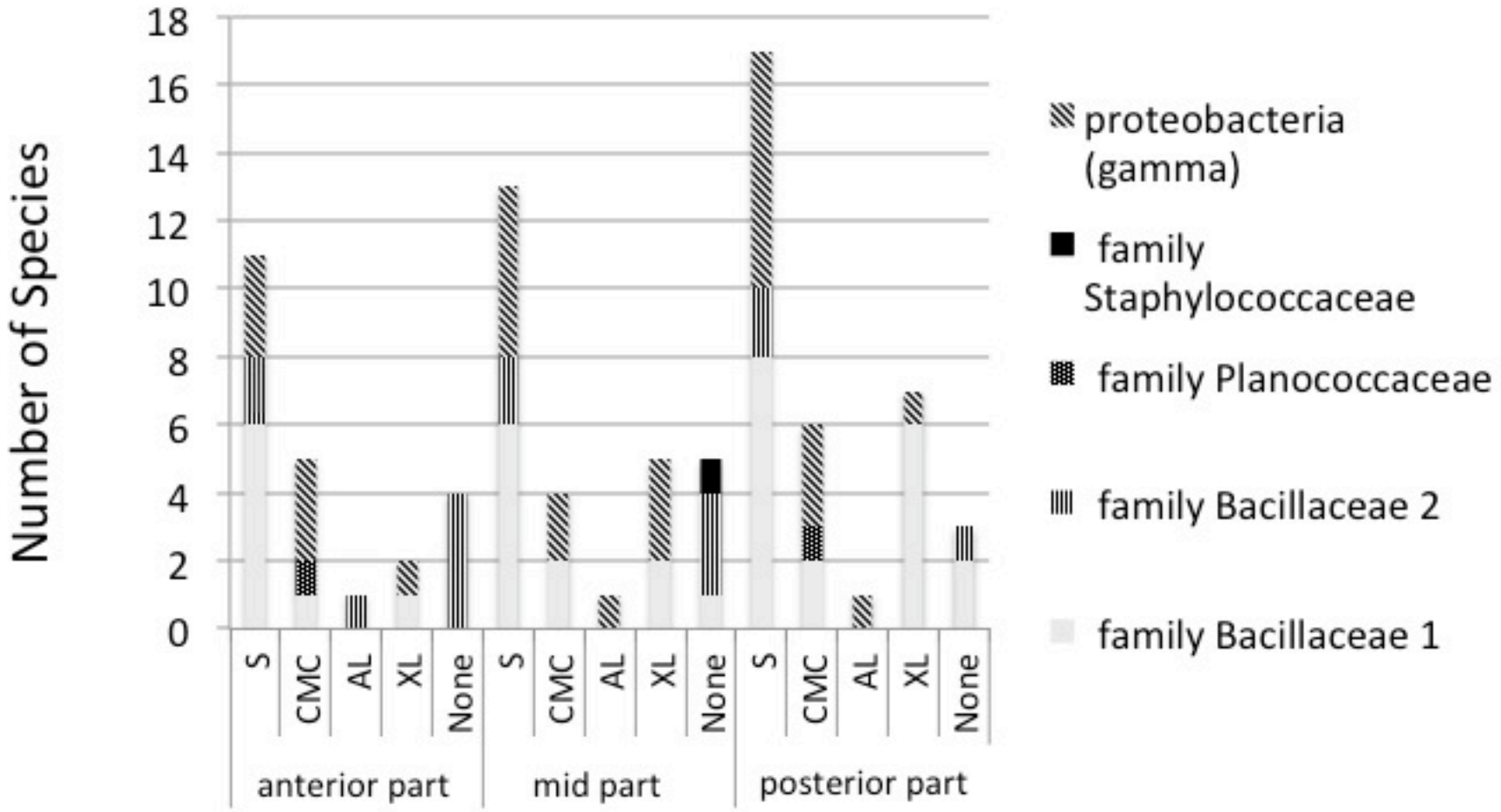

Fig.4 Number of the species of isolates degrading various polysaccharides
The species were divided into five groups as shown in Fig.3. S, CMC, AL, XL and None indicated starch degradation activity (S), CMC degradation activity (CMC), alginate degradation activity (AL), xylan degradation activity (XL) and no degradation activity (None), respectively. No isolates showed agarase activity.

Fig.5 Kudo

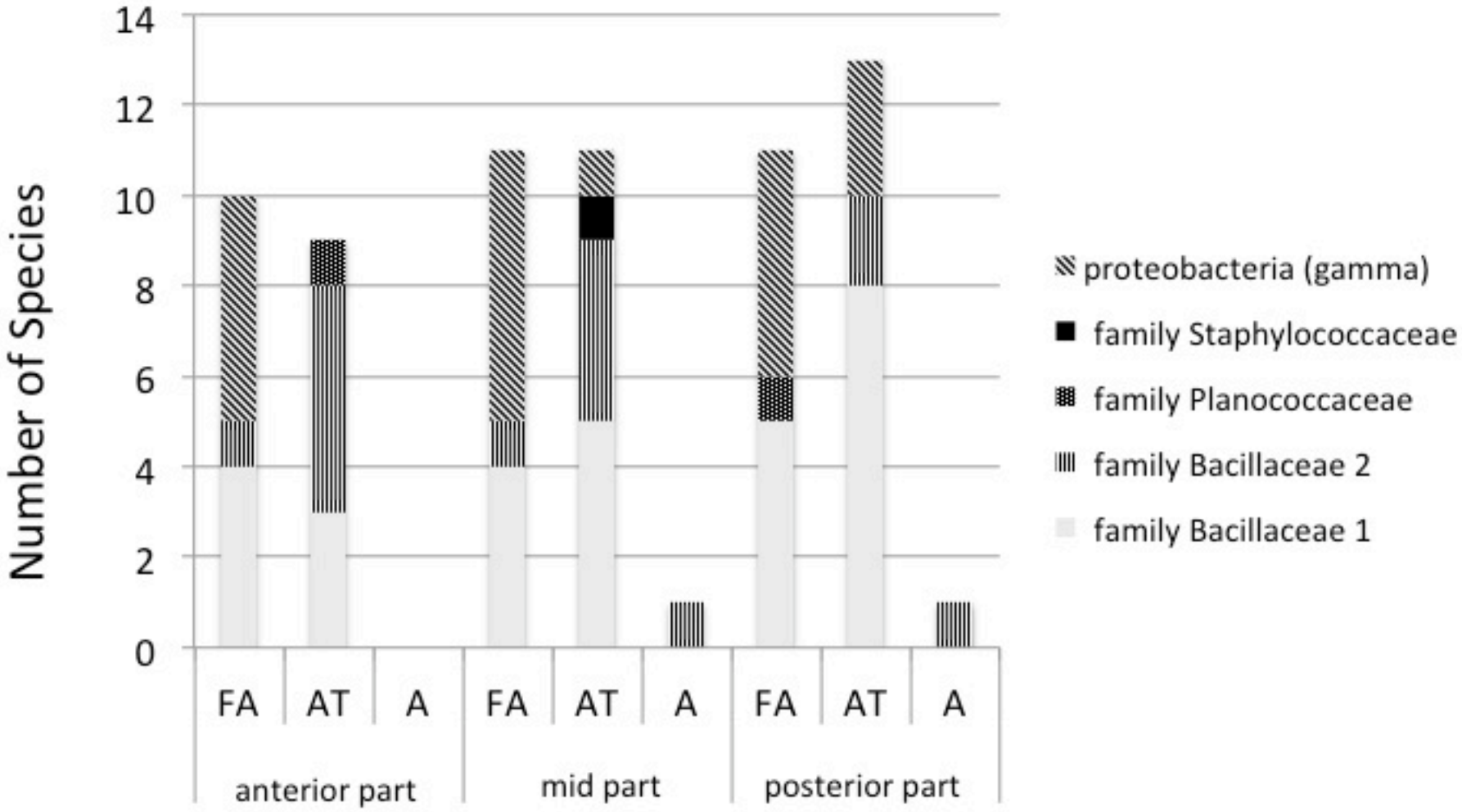

Fig.5 Number of the species of isolates classified by effect of oxygen on the growth
The species were divided into five groups as shown in Fig.3. Facultative anaerobic bacteria (FA) form colony in both aerobic and anaerobic cultivation. Anaerobic tolerant bacteria (AT) do not form colony in anaerobic 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.

Fig.6 kudo

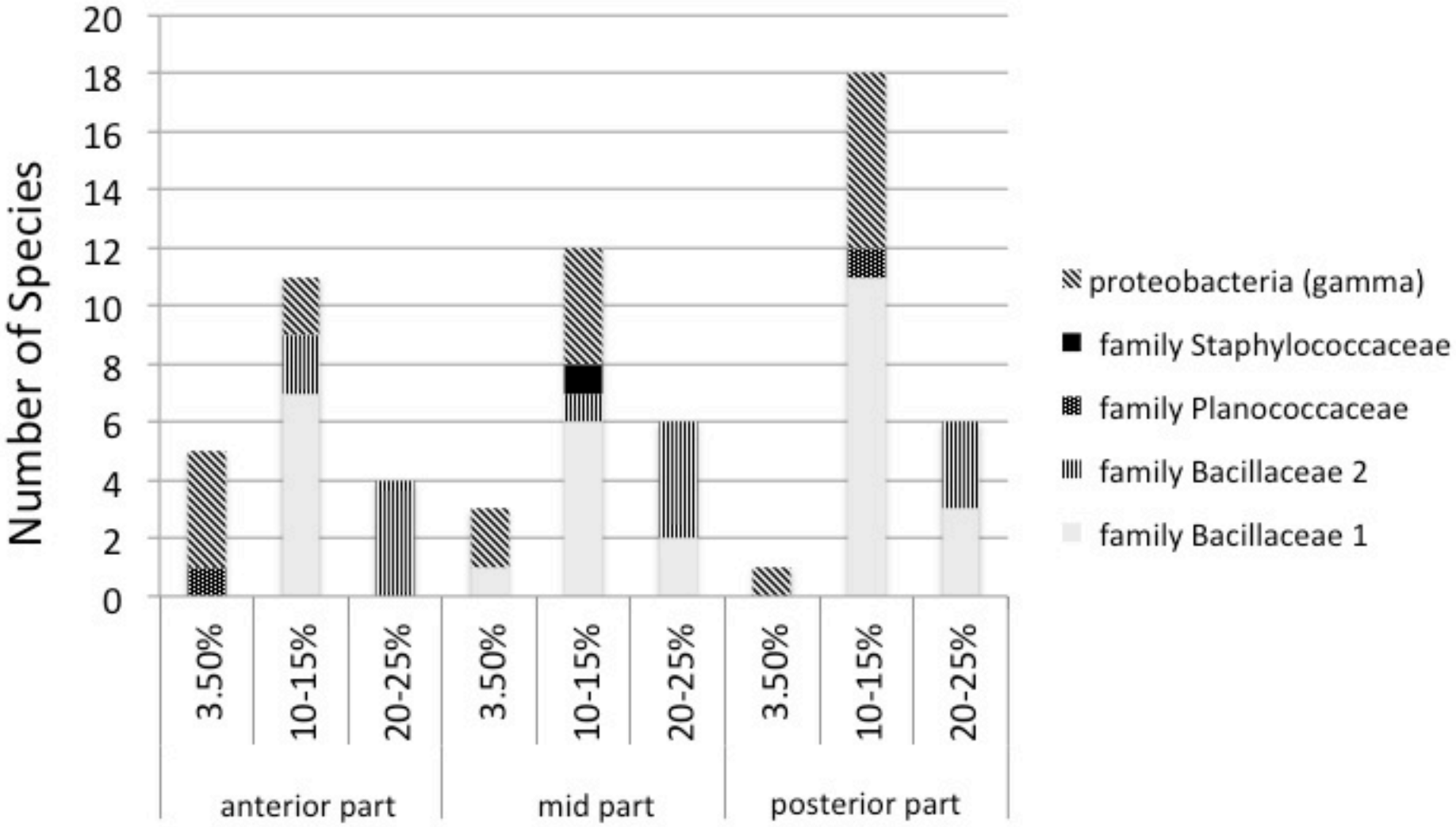

Fig. 6 Number of the species of isolates classified by effect of NaCl concentration on the growth
The species were divided into five groups as shown in Fig.3.

Table1 Number of the isolates obtained by differrent culture conditions

pH	Medium	Salinity	part of the intestine			subtotal
			anterior	mid	posterior	
pH 7	LB	3.5%	–	6	–	
	CMC	3.5%	1	3	–	
		10%	2	2	4	
	S	3.5%	2	3	1	
		10%	–	1	3	
	AL	3.5%	1	1	1	
		10%	4	2	7	
	XL	3.5%	2	3	1	
		10%	3	3	2	
subtotal			15	24	19	58
pH 10	CMC	3.5%	1	5	2	
		10%	–	–	2	
	S	3.5%	3	2	5	
		10%	–	–	–	
	AL	3.5%	2	–	1	
		10%	1	–	–	
	XL	3.5%	1	2	4	
		10%	1	–	2	
subtotal			9	9	16	34
Total			24	33	35	92

Table2 Phylogenetic affiliation for isolates (92 strains) from various parts of the intestine

phylum/class/family	genus	species /tentative species	part of the intestine		
			anterior	mid	posterior
phylum Firmicutes					
family Bacillaceae 1	*Bacillus* (21)	*Bacillus aerophilus*			+
		Bacillus aerophilus/Bacillus altitudinis	+		
		Bacillus altitudinis/Bacillus stratosphericus	+		
		Bacillus aquimaris			+
		Bacillus aryabhattai		+	
		Bacillus aurantiacus			+
		Bacillus clarkii/Bacillus polygoni		+	
		Bacillus clausii		+	
		Bacillus flexus	+		
		Bacillus hemicellulosilyticus			+
		*Bacillus horikoshii**	+		+
		Bacillus horti		+	
		*Bacillus hunanensis**	+		+
		Bacillus hunanensis/Bacillus oshimensis			+
		Bacillus hwajinpoensis			+
		Bacillus lehensis		+	
		*Bacillus licheniformis**		+	+
		Bacillus marisflavi		+	
		*Bacillus megaterium**	+	+	
		Bacillus neizhouensis	+		
		*Bacillus oshimensis**	+		+
		Bacillus pseudalcaliphilus			+
		Bacillus pumilus		+	
		*Bacillus vietnamensis**	+		+
		Bacillus wakoensis			+
family Bacillaceae 2	*Gracilibacillus* (1)	*Gracilibacillus dipsosauri*	+		
	Halobacillus (2)	*Halobacillus kuroshimensis**	+	+	+
		*Halobacillus trueperi**		+	+
	Oceanobacillus (3)	*Oceanobacillus kimchii*		+	
		Oceanobacillus oncorhynchi	+		
		Oceanobacillus sojae	+		
	Thalassobacillus (1)	*Thalassobacillus devorans*	+		
	Virgibacillus (3)	*Virgibacillus dokdonensis*	+		
		*Virgibacillus halodenitrificans**		+	+
		Virgibacillus marismortui		+	
family Planococcaceae	*Jeotgalibacillus* (1)	*Jeotgalibacillus campisalis**	+		+
family Staphylococcaceae	*Staphylococcus* (1)	*Staphylococcus warneri*		+	
phylum Proteobacteria					
class gamma	*Halomonas* (1)	*Halomonas meridiana*			+
	Marinobacter (1)	*Marinobacter alkaliphilus*			+
	Photobacterium (2)	*Photobacterium lutimaris*	+		
		*Photobacterium rosenbergii**		+	+
	Pseudomonas (3)	*Pseudomonas cedrina**		+	+
		*Pseudomonas libanensis**	+	+	+
		Pseudomonas synxantha		+	
	Vibrio (5)	*Vibrio agarivorans*			+
		Vibrio harveyi		+	
		Vibrio mediterranei	+		
		*Vibrio pomeroyi**	+	+	
		*Vibrio rotiferianus**	+		+

45 species

* indicated the species/tentative species found in multiple parts of the intestine

Supplementary Table 1

Isolate No.	Accession number of isolates	isolation medium	letters	part of the intestine	Accession nnumber of type strain	species/tentative species	Identities	Degrading activities on polysaacharides				Requiment of oxygen	Maximum NaCl concentration for growth	pH tolerance	phylum	family
								S	CMC	AL	XL					
C210	AB741781	LB(pH7,3.5%)	528	mid	EF114313	*Bacillus aryabhattai*	522/522 (100%)	+	+	-	-	FA	10	NE	firmicutes	family Bacillaceae 1
C212	AB741782	LB(pH7,3.5%)	818	mid	AF483624	*Bacillus marisflavi*	818/818 (100%)	-	-	-	+	AT	20	NE	firmicutes	family Bacillaceae 1
C214	AB741783	LB(pH7,3.5%)	623	mid	AJ009793	*Virgibacillus marismortui*	555/577 (96%)	-	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 2
C219	AB741784	LB(pH7,3.5%)	825	mid	AJ310149	*Halobacillus trueperi*	816/825 (98%)	+	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
C220	AB741785	LB(pH7,3.5%)	819	mid	L37603	*Staphylococcus warneri*	819/819 (100%)	-	-	-	-	AT	15	NE	firmicutes	family Staphylococcaceae
C221	AB741786	LB(pH7,3.5%)	509	mid	AJ491290	*Vibrio pomeroyi*	503/509 (98%)	+	+	-	-	FA	3.5	ALK	proteobacteria	gamma
C234	AB741787	CMC(pH10,10%)	817	posterior	HM054473	*Bacillus hunanensis*	816/817 (99%)	+	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 1
C235	AB741788	CMC(pH10,10%)	813	posterior	AB125942	*Marinobacter alkaliphilus*	807/813 (99%)	+	-	-	-	AT	15	ALK	proteobacteria	gamma
C236	AB741789	CMC(pH10,3.5%)	594	anterior	AY190535	*Jeotgalibacillus campisalis*	590/594 (99%)	-	+	-	-	AT	3.5	ALK	firmicutes	family Planococcaceae
C240	AB741790	CMC(pH10,3.5%)	499	mid	X76440	*Bacillus clausii*	497/499 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
C241	AB741791	CMC(pH10,3.5%)	589	mid	D87035	*Bacillus horti*	585/589 (99%), Gaps = 1/589 (0%)	+	-	-	-	AT	3.5	ALK	firmicutes	family Bacillaceae 1
C242	AB741792	CMC(pH10,3.5%)	540	mid	AJ842344	*Photobacterium rosenbergii*	537/540 (99%)	+	-	-	-	FA	3.5	ALK	proteobacteria	gamma
C243	AB741793	CMC(pH10,3.5%)	797	mid	D87035	*Bacillus horti*	791/797 (99%), Gaps = 2/797 (0%)	+	-	-	-	AT	3.5	ALK	firmicutes	family Bacillaceae 1
C245	AB741794	CMC(pH10,3.5%)	812	mid	GU784860	*Oceanobacillus kimchii*	807/812 (99%)	-	-	-	-	FA	20	ALK	firmicutes	family Bacillaceae 2
C246	AB741795	CMC(pH10,3.5%)	574	posterior	AB188090	*Bacillus oshimensis*	571/574 (99%)	-	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
C247	AB741796	CMC(pH10,3.5%)	427	posterior	AJ492830	*Pseudomonas cedrina*	425/427 (99%)	+	-	-	-	FA	15	ALK	proteobacteria	gamma
C254	AB741797	AL(pH10,10%)	453	anterior	AJ717299	*Thalassobacillus devorans*	437/453 (96%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
C258	AB741798	AL(pH10,3.5%)	621	anterior	EU925618	*Bacillus neizhouensis*	609/616 (98%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
C259	AB741799	AL(pH10,3.5%)	715	anterior	AJ316187	*Vibrio rotiferianus*	691/699 (98%)	-	+	-	-	FA	3.5	ALK	proteobacteria	gamma
C263	AB741800	AL(pH10,3.5%)	524	posterior	AF057645	*Pseudomonas libanensis*	523/524 (99%)	+	+	-	-	AT	10	ALK	proteobacteria	gamma
C265	AB741801	XL(pH10,10%)	535	anterior	AB188090	*Bacillus oshimensis*	530/535 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
C270	AB741802	XL(pH10,10%)	565	posterior	HM054473	*Bacillus hunanensis*	565/565 (100%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
C271	AB741803	XL(pH10,10%)	764	posterior	AJ605773	*Bacillus aurantiacus*	732/759 (96%), Gaps = 6/759 (0%)	+	-	-	+	AT	20	ALK	firmicutes	family Bacillaceae 1
C277	AB741804	XL(pH10,3.5%)	677	anterior	AB043865	*Bacillus horikoshii*	675/677 (99%), Gaps = 1/677 (0%)	+	-	-	-	AT	10	ALK	firmicutes	family Bacillaceae 1
C280	AB741805	XL(pH10,3.5%)	544	mid	D84025	*Pseudomonas synxantha*	540/544 (99%)	+	-	+	+	FA	10	ALK	proteobacteria	gamma
C281	AB741806	XL(pH10,3.5%)	475	mid	X76444/AB292819	*Bacillus clarkii/Bacillus polygoni*	474/475 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
C285	AB741807	XL(pH10,3.5%)	581	posterior	AY190535	*Jeotgalibacillus campisalis*	581/583 (99%), Gaps = 1/583 (0%)	-	+	-	-	FA	10	ALK	firmicutes	family Planococcaceae
C287	AB741808	XL(pH10,3.5%)	637	posterior	HM054473/AB188090	*Bacillus hunanensis/Bacillus oshimensis*	634/637 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
C288	AB741809	XL(pH10,3.5%)	772	posterior	X76449	*Bacillus pseudalcaliphilus*	743/760 (97%)	+	-	-	+	FA	10	ALK	firmicutes	family Bacillaceae 1
C291	AB741810	XL(pH10,3.5%)	521	posterior	AB043851	*Bacillus wakoensis*	521/521 (100%)	-	-	-	+	FA	15	ALK	firmicutes	family Bacillaceae 1
C295	AB741811	S(pH10,3.5%)	515	anterior	DQ534014	*Photobacterium lutimaris*	512/515 (99%)	+	-	-	-	FA	3.5	ALK	proteobacteria	gamma
C298	AB741812	S(pH10,3.5%)	816	anterior	HM054473	*Bacillus hunanensis*	815/816 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
C302	AB741813	S(pH10,3.5%)	411	anterior	X74710	*Vibrio mediterranei*	407/411 (99%)	-	+	-	-	FA	3.5	ALK	proteobacteria	gamma
C304	AB741814	S(pH10,3.5%)	540	mid	AJ842344	*Photobacterium rosenbergii*	535/540 (99%)	+	-	-	-	FA	10	ALK	proteobacteria	gamma
C305	AB741815	S(pH10,3.5%)	683	mid	X76440	*Bacillus clausii*	673/677 (99%)	+	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 1
C307	AB741816	S(pH10,3.5%)	550	posterior	AB043846	*Bacillus hemicellulosilyticus*	547/550 (99%)	+	+	-	+	AT	10	ALK	firmicutes	family Bacillaceae 1
C309	AB741817	S(pH10,3.5%)	565	posterior	AJ842344	*Photobacterium rosenbergii*	560/565 (99%)	-	+	-	-	FA	10	ALK	proteobacteria	gamma
C310	AB741818	S(pH10,3.5%)	623	posterior	AF057645	*Pseudomonas libanensis*	619/623 (99%)	+	-	-	-	AT	15	ALK	proteobacteria	gamma
C312	AB741819	S(pH10,3.5%)	516	posterior	AJ310647	*Vibrio agarivorans*	505/516 (97%)	+	-	+	-	FA	10	ALK	proteobacteria	gamma
C313	AB741820	S(pH10,3.5%)	388	posterior	AB043865	*Bacillus horikoshii*	388/388 (100%)	+	-	-	-	FA	10	ALK	firmicutes	family Bacillaceae 1
C317	AB741821	CMC(pH7,10%)	580	anterior	AB099708	*Bacillus vietnamensis*	579/580 (99%)	+	-	-	-	FA	10	NE	firmicutes	family Bacillaceae 1
C318	AB741822	CMC(pH7,10%)	780	anterior	AJ831844/AJ831842	*Bacillus aerophilus/Bacillus altitudinis*	780/780 (100%)	-	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
C324	AB741823	CMC(pH7,10%)	569	mid	AY543169	*Virgibacillus halodenitrificans*	568/569 (99%)	-	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 2
C327	AB741824	CMC(pH7,10%)	811	mid	AB195680	*Halobacillus kuroshimensis*	808/811 (99%), Gaps = 2/811 (0%)	+	-	-	-	AT	25	NE	firmicutes	family Bacillaceae 2
C333	AB741825	CMC(pH7,10%)	679	posterior	AY543169	*Virgibacillus halodenitrificans*	675/676 (99%)	-	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 2
C334	AB741826	CMC(pH7,10%)	586	posterior	CP000002	*Bacillus licheniformis*	580/580 (100%)	+	-	-	-	FA	15	NE	firmicutes	family Bacillaceae 1
C335	AB741827	CMC(pH7,10%)	808	posterior	AJ310149	*Halobacillus trueperi*	806/808 (99%)	+	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 2
C336	AB741828	CMC(pH7,10%)	740	posterior	AF483625	*Bacillus aquimaris*	735/740 (99%)	+	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
C339	AB741829	AL(pH7,10%)	589	anterior	AJ640134	*Oceanobacillus oncorhynchi*	577/589 (97%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
C341	AB741830	AL(pH7,10%)	558	anterior	AJ831842/AJ831841	*Bacillus altitudinis/Bacillus stratosphericus*	558/558 (100%)	-	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
C344	AB741831	AL(pH7,10%)	768	anterior	AJ831844/AJ831842	*Bacillus aerophilus/Bacillus altitudinis*	768/768 (100%)	-	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
C347	AB741832	AL(pH7,10%)	704	anterior	AY822043	*Virgibacillus dokdonensis*	691/704 (98%)	-	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 2

C350	AB741833	AL(pH7,10%)	823	mid	AB195680	*Halobacillus kuroshimensis*	816/820 (99%), Gaps = 3/820 (0%)	+	-	-	-	A	20	NE	firmicutes	family Bacillaceae 2
C355	AB741834	AL(pH7,10%)	684	mid	AB195680	*Halobacillus kuroshimensis*	682/685 (99%), Gaps = 2/685 (0%)	+	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 2
C358	AB741835	AL(pH7,10%)	753	posterior	AF541966	*Bacillus hwajinpoensis*	746/749 (99%), Gaps = 1/749 (0%)	-	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
C359	AB741836	AL(pH7,10%)	528	posterior	CP000002	*Bacillus licheniformis*	528/528 (100%)	+	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
C360	AB741837	AL(pH7,10%)	557	posterior	AB099708	*Bacillus vietnamensis*	557/557 (100%)	+	-	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
C361	AB741838	AL(pH7,10%)	665	posterior	AY543169	*Virgibacillus halodenitrificans*	663/665 (99%)	-	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 2
C362	AB741839	AL(pH7,10%)	813	posterior	AB195680	*Halobacillus kuroshimensis*	810/814 (99%), Gaps = 2/814 (0%)	+	-	-	-	A	25	NE	firmicutes	family Bacillaceae 2
C365	AB741840	AL(pH7,10%)	772	posterior	AF483625	*Bacillus aquimaris*	760/772 (98%)	+	-	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
C367	AB741841	AL(pH7,10%)	520	posterior	AF541966	*Bacillus hwajinpoensis*	515/520 (99%)	-	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 1
C370	AB741842	XL(pH7,10%)	420	anterior	AB101591	*Gracilibacillus dipsosauri*	420/420 (100%)	+	-	+	-	AT	15	ALK	firmicutes	family Bacillaceae 2
C375	AB741843	XL(pH7,10%)	598	anterior	AB473561	*Oceanobacillus sojae*	596/598 (99%)	-	-	-	-	AT	20	ALK	firmicutes	family Bacillaceae 2
C377	AB741844	XL(pH7,10%)	760	anterior	AB195680	*Halobacillus kuroshimensis*	758/761 (99%), Gaps = 2/761 (0%)	+	-	-	-	FA	25	ALK	firmicutes	family Bacillaceae 2
C379	AB741845	XL(pH7,10%)	533	mid	CP000002	*Bacillus licheniformis*	531/532 (99%)	+	-	-	-	FA	15	ALK	firmicutes	family Bacillaceae 1
C380	AB741846	XL(pH7,10%)	535	mid	X76440	*Bacillus clausii*	534/535 (99%)	-	-	-	-	AT	25	NE	firmicutes	family Bacillaceae 1
C381	AB741847	XL(pH7,10%)	836	mid	AB195680	*Halobacillus kuroshimensis*	831/837 (99%), Gaps = 2/837 (0%)	+	-	-	-	A	20	ALK	firmicutes	family Bacillaceae 2
C392	AB741848	XL(pH7,10%)	655	posterior	CP000002	*Bacillus licheniformis*	653/655 (99%)	+	-	-	+	FA	15	NE	firmicutes	family Bacillaceae 1
C397	AB741849	XL(pH7,10%)	568	posterior	AJ842344	*Photobacterium rosenbergii*	565/569 (99%), Gaps = 1/569 (0%)	+	-	-	-	FA	15	NE	proteobacteria	gamma
C416	AB741850	S(pH7,10%)	711	mid	CP000002	*Bacillus licheniformis*	710/711 (99%)	+	-	-	-	FA	15	NE	firmicutes	family Bacillaceae 1
C417	AB741851	S(pH7,10%)	672	posterior	CP000002	*Bacillus licheniformis*	671/672 (99%)	+	+	-	-	FA	15	NE	firmicutes	family Bacillaceae 1
C431	AB741852	S(pH7,10%)	777	posterior	AY543169	*Virgibacillus halodenitrificans*	777/777 (100%)	-	-	-	-	AT	20	NE	firmicutes	family Bacillaceae 2
C435	AB741853	CMC(pH7,3.5%)	706	anterior	AJ491290	*Vibrio pomeroyi*	699/706 (99%), Gaps = 1/706 (0%)	+	+	-	-	FA	3.5	ALK	proteobacteria	gamma
C442	AB741854	CMC(pH7,3.5%)	531	mid	AJ491290	*Vibrio pomeroyi*	525/531 (98%)	+	+	-	-	FA	3.5	ALK	proteobacteria	gamma
C444	AB741855	CMC(pH7,3.5%)	421	mid	D16273	*Bacillus megaterium*	417/421 (99%)	+	+	-	-	AT	10	NE	firmicutes	family Bacillaceae 1
C446	AB741856	CMC(pH7,3.5%)	568	mid	AY876289	*Bacillus pumilus*	568/569 (99%)	-	-	-	+	FA	10	NE	firmicutes	family Bacillaceae 1
C460	AB741858	AL(pH7,3.5%)	477	anterior	AJ491290	*Vibrio pomeroyi*	471/477 (98%)	+	+	-	-	FA	3.5	ALK	proteobacteria	gamma
C463	AB741859	AL(pH7,3.5%)	462	mid	AF057645	*Pseudomonas libanensis*	461/462 (99%)	+	-	-	-	AT	10	ALK	proteobacteria	gamma
C466	AB741860	AL(pH7,3.5%)	664	posterior	AJ316187	*Vibrio rotiferianus*	662/664 (99%)	+	+	-	-	FA	3.5	ALK	proteobacteria	gamma
C471	AB741861	XL(pH7,3.5%)	544	anterior	AF057645	*Pseudomonas libanensis*	542/544 (99%)	-	-	-	+	FA	15	NE	proteobacteria	gamma
C475	AB741862	XL(pH7,3.5%)	655	anterior	AJ316187	*Vibrio rotiferianus*	654/655 (99%)	+	+	-	-	FA	15	ALK	proteobacteria	gamma
C483	AB741863	XL(pH7,3.5%)	673	mid	AF057645	*Pseudomonas libanensis*	671/673 (99%)	+	-	-	+	AT	15	ALK	proteobacteria	gamma
C484	AB741864	XL(pH7,3.5%)	542	mid	AJ492830	*Pseudomonas cedrina*	540/542 (99%)	+	-	-	-	FA	15	ALK	proteobacteria	gamma
C487	AB741865	XL(pH7,3.5%)	535	mid	AY750575	*Vibrio harveyi*	529/535 (98%)	-	+	-	-	FA	15	ALK	proteobacteria	gamma
C494	AB741866	XL(pH7,3.5%)	761	posterior	AJ831844	*Bacillus aerophilus*	761/761 (100%)	-	-	-	+	AT	15	NE	firmicutes	family Bacillaceae 1
C503	AB741867	S(pH7,3.5%)	749	anterior	D16273	*Bacillus megaterium*	747/749 (99%)	+	-	-	-	FA	10	NE	firmicutes	family Bacillaceae 1
C506	AB741868	S(pH7,3.5%)	540	anterior	AB021185	*Bacillus flexus*	538/540 (99%)	+	+	-	-	AT	15	NE	firmicutes	family Bacillaceae 1
C512	AB741869	S(pH7,3.5%)	556	mid	AF057645	*Pseudomonas libanensis*	554/556 (99%)	+	-	-	+	FA	10	NE	proteobacteria	gamma
C520	AB741870	S(pH7,3.5%)	563	mid	AJ492830	*Pseudomonas cedrina*	561/564 (99%), Gaps = 1/564 (0%)	-	-	-	+	FA	15	NE	proteobacteria	gamma
C521	AB741871	S(pH7,3.5%)	669	mid	AY793550	*Bacillus lehensis*	668/669 (99%)	+	-	-	-	AT	15	ALK	firmicutes	family Bacillaceae 1
C528	AB741872	S(pH7,3.5%)	578	posterior	AF057645	*Pseudomonas libanensis*	576/578 (99%)	-	+	-	+	FA	10	NE	proteobacteria	gamma
C538	AB741873	S(pH7,10%)	746	posterior	AJ306891	*Halomonas meridiana*	746/746 (100%)	+	-	-	-	AT	15	ALK	proteobacteria	gamma

Abbreviations: S, starch; CMC, carboxymethyl cellulose sodium salt; AL, alginate; XL, xylan; FA, facultative anaerobic bacteria; AT, anaerobic tolerant bacteria; A, aerobic bacteria;NE, neutrophilic bacteria; ALK, alkaliphilic/alkali-tolerant bacteria . Display of more than one species in the column of nearest type strain species indicated the same idenntity in the comparison range.