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PII: S0023-6438(17)30613-8

DOI: 10.1016/j.lwt.2017.08.043

Reference: YFSTL 6465

To appear in: *LWT - Food Science and Technology*

Received Date: 1 June 2017

Revised Date: 2 August 2017

Accepted Date: 14 August 2017

Please cite this article as: Kim, S., Lee, M.-J., Lee, J.-J., Choi, S.H., Kim, B.-S., Analysis of microbiota of the swimming crab (*Portunus trituberculatus*) in South Korea to identify risk markers for foodborne illness, *LWT - Food Science and Technology* (2017), doi: 10.1016/j.lwt.2017.08.043.

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1 # Analysis of microbiota of the swimming crab (*Portunus trituberculatus*) in
2 # South Korea to identify risk markers for foodborne illness

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20 ## Abstract

21

22 The swimming crab *Portunus trituberculatus* is one of the most popular seafoods in South
23 Korea. Despite yearly reports of foodborne illness caused by swimming crab consumption,
24 the microbiota in the whole body of the crab has not been fully characterized. A total of 60
25 crabs were collected from different sites in the Yellow Sea in the spring and autumn, and the
26 crab microbiota was analyzed by 16S rRNA pyrosequencing. Crab microbial diversity was
27 higher in the autumn than in the spring. *Psychrobacter, Vagococcus, Carnobacterium,*
28 *Lactococcus,* and *Streptococcus* were dominant genera in the spring, whereas *Roseovarius*
29 and various other genera were dominant in the autumn. The composition of the microbiota
30 differed significantly between spring and autumn ($p < 0.01$). The relative abundances of
31 potential pathogens, including *Lactococcus garvieae,* in crabs were higher in spring than in
32 autumn. Although further studies are necessary to clarify the pathogenicity of these potential
33 pathogens, the potential risk of foodborne illness by crab intake could be higher in spring
34 than autumn. This study extends our knowledge of potential foodborne illness according to
35 microbiota and may improve the management of crab products.

36

37 ***Keywords:*** Swimming crab; *Portunus trituberculatus*; Microbiota; Potential pathogens

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38 **1. Introduction**

39

40 The swimming crab (*Portunus trituberculatus*) is widely distributed in the Indian and West
41 Pacific Oceans and is an important commercial species in Southeast and East Asia (FAO,
42 2013). The global production of *P. trituberculatus* has increased since the 1970s, and 503,855
43 t was captured in 2013 to accommodate increased consumption (FAO, 2013). The daily
44 consumption rate in South Korea has been particularly high in recent years, and the
45 swimming crab is one of the most highly consumed seafoods in South Korea (Moon, Kim,
46 Choi, Yu, & Choi, 2009; Zhang et al., 2014).

47 Marinated crab (Gejang) is a popular food in South Korea, and pathogenic microorganisms
48 in the crab microbiota can be causative agents of foodborne illness. Bacterial foodborne
49 illness occasionally occurs by crab consumption around the world (Kwon et al., 2000;
50 Matulkova et al., 2013; Park, Kim, Won, & Seo, 2008). Therefore, it is necessary to identify
51 the bacterial members in swimming crabs to understand the potential effects on human health
52 and prevent foodborne illness caused by crab consumption. Some studies have analyzed the
53 microbiota in crab gills and guts to identify symbionts and potential pathogens (Givens,
54 Burnett, Burnett, & Hollibaugh, 2013; Zhang et al., 2016). However, little is known about the
55 bacterial communities in the whole body of the swimming crab. Microorganisms in the whole
56 body of the crab could be transmitted to humans during handling, and whole bodies are often
57 consumed in marinated form. Therefore, the analysis of crab microbiota using the whole
58 body is necessary to understand and prevent foodborne illness.

59 Here, we analyzed the microbiota in the whole body of crabs obtained from different
60 sampling sites with high production in Korea using high-throughput sequencing. The
61 microbial compositions of crabs were compared among sampling regions and seasons. In

62 addition, we identified potential pathogens. The study results extend our understanding of
63 food poisoning by crabs and may improve the management of products.
64

65 **2. Material and methods**

66

67 *2.1. Crab sample collection*

68

69 A total of 60 swimming crabs (*P. trituberculatus*) were collected from 6 sites in the Yellow
70 sea in Korea in May of 2015 (spring) and October of 2014 (autumn) (Fig. 1). Crab
71 consumption is highest in the spring and autumn in South Korea. The areas with the highest
72 production in Korea were chosen as sampling sites (Oh, 2011). Five crabs were randomly
73 collected at each site and were immediately transported in an icebox to the laboratory.
74

75 *2.2. Metagenomic DNA extraction*

76

77 To extract metagenomic DNA from crab samples, bacteria were detached and collected
78 using SPINDLE, as previously described (Kim et al., 2012; Lee, Lee, Chung, Choi, & Kim,
79 2016). The crab shells, pincers, and legs were separated, placed in sterile sample bags with
80 225 ml of buffered peptone water, and treated with SPINDLE for 2 min at 7000 × *g*. To
81 remove PCR inhibitors, the extracted DNA was cleaned using the PowerClean Pro DNA
82 Clean-Up Kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's
83 protocol. The concentration of purified DNA was determined using a NanoVue
84 spectrophotometer (GE Healthcare, Waukesha, WI, USA).
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2.3. Pyrosequencing

The hypervariable regions (V1–V3) of 16S rRNA genes were amplified from extracted DNA using barcoded primers (Supplementary Table S1), as described previously (Hur et al., 2011; Lee et al., 2016). Amplification was performed in a final volume of 50 μL containing 10× *Taq* buffer, dNTP mixture (Takara, Shiga, Japan), 10 μM each barcoded fusion primer, and 2 U of *Taq* polymerase (*ExTaq*, Takara) using a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA). After initial denaturation at 94 °C for 5 min, the product was amplified by 30 cycles of denaturation (30 s, 94 °C), primer annealing (30 s, 55 °C), and extension (30 s, 72 °C), with a final extension step of 7 min at 72 °C. The PCR product was confirmed by 2% agarose gel electrophoresis and visualized under a Gel Doc system (Bio-Rad; Supplementary Fig. S1). The amplified products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Equimolar concentrations of each amplicon were pooled and sequenced using the 454 GS FLX Titanium XL Plus (Roche, Branford, CT, USA) following the manufacturer's instructions.

2.4. Sequence data analysis

Sequence reads obtained from pyrosequencing were analyzed according to previous methods (Jeon, Chun, & Kim, 2013). Briefly, sequence reads from each sample were sorted by unique barcodes and low-quality reads (average quality score < 25 or read length < 300 bp) were removed. The primer sequences were trimmed by pairwise alignment, and sequences were clustered with a 97% similarity threshold to correct sequencing errors. Representative

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110 sequences in each cluster were selected for taxonomic assignment and identified by BLAST
111 searches against the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net). Possible chimeric
112 sequences were removed using the UCHIME program (Edgar, Haas, Clemente, Quince, &
113 Knight, 2011). The number of sequencing reads in each sample was normalized by random
114 subsampling and diversity indices were calculated using the mothur program (Schloss et al.,
115 2009). Microbiota were compared among samples using a principal coordinate analysis
116 (PCoA) based on unweighted UniFrac distances. The sequence reads in this study were
117 submitted to the EMBL SRA database under study number PRJEB21988
118 (https://www.ebi.ac.uk/ena/data/view/PRJEB21988).

119

120 *2.5. Statistical analysis*

121

122 Differences in diversity indices and microbes among samples were analyzed by Mann–
123 Whitney U tests implemented in R (Mann & Whitney, 1947). Results are presented as means
124 ± standard deviation (SD). Differences between samples were considered significant when p
125 < 0.05.

126

127 **3. Results**

128

129 *3.1. Comparison of microbial diversity among crab samples*

130

131 A total of 933,238 reads (average 14,519 reads per spring sample and 16,589 reads per
132 autumn sample) were analyzed after quality filtering (Table 1). The diversity indices for
133 samples were compared after read numbers were normalized by random subsampling. The

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134 average Good's coverage was 0.94 ± 0.04. The Shannon diversity index was higher for crabs
135 collected in autumn than in spring (Fig. 2; $p < 0.001$). The microbiota for crabs exhibited the
136 highest diversity at site 2 and the lowest diversity at site 3 in the spring. Microbial diversity
137 was highest for in crabs from site 6 and lowest for crabs at site 2 in the autumn. A significant
138 difference in diversity between sampling sites within the same sampling season was only
139 detected between sites 2 and 3 in the spring ($p = 0.032$). There were no significant differences
140 in microbial diversity among sampling sites in the autumn ($p > 0.05$).
141

142 *3.2. Comparison of the microbial composition in crabs from different sampling seasons and*
143 *sites*
144

145 The composition of the crab microbiota differed significantly between spring and autumn
146 at the phylum level ($p < 0.001$; Fig. 3). Proteobacteria and Firmicutes were dominant in the
147 microbiota of all crabs in the spring, whereas diverse phyla were detected in the microbiota of
148 crabs in the autumn. The relative abundance of Firmicutes was higher in spring samples than
149 in autumn samples (Fig. 3A; $p < 0.001$). The relative abundances of other dominant phyla,
150 including Proteobacteria, Bacteroidetes, Actinobacteria, and Tenericutes, were higher in
151 autumn samples than in spring samples ($p < 0.001$).
152 Firmicutes was the dominant phylum in crabs from sites S3 (mean, 94.6%), S6 (91.79%),
153 and S2 (68.21%), whereas Proteobacteria was dominant at S5 (65.67%), S1 (64.55%), and S4
154 (63.08%) in the spring (Fig. 3B). Various microbial phyla were detected in crabs in the
155 autumn. Proteobacteria was the dominant phylum (57.22% to 80.22%) at all sampling sites in
156 the autumn. The highest proportion of Proteobacteria was detected in crabs from site A5, and
157 the lowest proportion was detected at site A3 ($p < 0.05$). Bacteroidetes was the next most

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158 dominant phylum at all sampling sites, except A2 (7.5% to 17.73%). Although the proportion
159 of Bacteroidetes was highest at site A6, the difference between sampling sites was not
160 significant ($p > 0.05$). Tenericutes was the second most abundant phylum at A2 (16.56%).
161 The third most dominant phylum was variable among sampling sites in the autumn.

162 The crab microbiota was further examined at the genus level. *Psychrobacter* was the
163 dominant genus in samples dominated by Proteobacteria (61.80% in S5, 58.96% in S1, and
164 56.94% in S4) in the spring ($p < 0.01$ for the difference between Proteobacteria-dominant
165 sites and Firmicutes-dominant sites; Fig. 4A). Various genera were dominant in Firmicutes-
166 dominant samples. *Lactococcus* (34.46%) and *Shewanella* (10.67%) were the dominant
167 genera at S2 ($p < 0.01$ in comparison with other sites, except S6); *Carnobacterium* (37.69%)
168 and *Vagococcus* (42.86%) were dominant genera at S3 ($p < 0.05$ in comparison with other
169 sites); *Lactococcus* (29.89%) and *Streptococcus* (40.11%) were dominant genera at S6 ($p <$
170 0.05 in comparison with other sites, except S1 and S4). The composition of genera was more
171 diverse in autumn than in spring. However, Proteobacteria was dominant in all crab samples
172 in the autumn, and *Psychrobacter* was the dominant genus only at site A5 (35.71%).
173 *Roseovarius* within Alphaproteobacteria was the dominant genus in the autumn; its relative
174 abundance was highest at site A1 (24.67%). *Arcobacter* (18.59%) was the dominant genus at
175 site A4 ($p < 0.05$ compared with other sites). In addition, uncultured bacteria were more
176 common in autumn (20.17% to 40.61%) than in spring (0.15% to 8.47%). The dominant
177 genera in the crab microbiota (mean proportion >1% at each site) differed significantly
178 between the spring and autumn ($p < 0.01$; Supplementary Fig. S2). The relative abundances
179 of *Psychrobacter*, *Carnobacterium*, *Vagococcus*, *Lactococcus, Streptococcus*, and *Shewanella*
180 were higher in spring than autumn, whereas those of *Roseovarius*, *Pelagicola*, *Arcobacter*,

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181 *Formosa*, *Ilumatobacter*, uncultured *Clostridiales*, uncultured *Rhodobacteraceae*, and
182 uncultured *Lumbricoplasmataceae* were higher in autumn than spring.
183 The difference in crab microbiota between sampling seasons was also investigated by a
184 principal coordinates analysis (PCoA) based on unweighted UniFrac distances (Fig. 5). The
185 crab microbiota was clearly separated between spring and autumn samples. The microbiota
186 was more similar among sampling sites for autumn crabs than spring crabs. The microbiota
187 of crabs from A5 was different from those of crabs from other sites in the autumn. However,
188 the spring crabs were subdivided into two groups, a cocci-dominated group (S2, 24, and S5)
189 and a *Psychrobacter*-dominated group (S1, S3, and S6).
190
191 *3.3. Potential pathogens in swimming crabs*
192
193 The frequencies of 11 species related to foodborne illness were analyzed to identify
194 potential pathogens in the swimming crab (Daskalov, 2006; Iwamoto, Ayers, Mahon, &
195 Swerdlow, 2010; Scallan et al., 2011) (Table 2). The sum of the frequencies of foodborne
196 pathogens was higher in spring (average 11.48 ± 14.75%) than in autumn (0.03 ± 0.04%), and
197 the presence of pathogenic bacteria differed depending on the season. The pathogenic species
198 *Aeromonas hydrophila* was detected only in the spring, whereas *Escherichia coli* and
199 *Staphylococcus epidermidis* were detected in the autumn. The average proportion of
200 *Lactococcus garvieae,* a potential foodborne pathogen, was higher in spring (10.81%) than in
201 autumn (0.02%).
202 The detection of pathogenic bacteria also differed among sampling sites within the same
203 season. In the spring, the proportion of pathogenic bacteria was relatively high at site 2
204 (36.15%) and site 6 (27.81%) compared to other sites (0.1% to 2.32%) owing to the

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205 abundance of *L. garvieae* at these sites. Although *A. hydrophila* was detected in all spring
206 crab samples except site 6, it was more abundant at site 2 (2.60%) than at other sites. In the
207 autumn, the relative abundances of foodborne pathogens were lower at site 2 (0.02%), site 3
208 (not detected), and site 5 (0.02%) than at other sites (0.07% to 0.27%).

209

210 **4. Discussion**

211

212 In this study, the swimming crab microbiota was analyzed and compared among seasons
213 and sites in the Yellow sea in South Korea. The diversity and composition of the crab
214 microbiota differed significantly between spring and autumn (Fig. 2 and 3). The differences
215 in microbiota among sampling sites were relatively small compared to the differences
216 between seasons. These results indicated that the crab microbiota is strongly influenced by
217 season, and accordingly might be related to differences in water temperature. The
218 temperature of the Yellow Sea is highest in August (24.8–25.1 °C) and lowest in February
219 (3.0–4.1 °C), while the salinity is fairly steady throughout the year (29.3–31.5‰) (KOOFS,
220 2015). The microbiota in spring crabs was influenced by the relatively low temperature
221 during the winter, whereas that in autumn crabs was influenced by the higher temperature
222 during the summer (Moisander, Sexton, & Daley, 2015; Pinto, Schroeder, Lunn, Sloan, &
223 Raskin, 2014). Therefore, the microbial diversity was higher in autumn crabs than in spring
224 crabs owing to the higher temperature, which promotes the growth of diverse microbes. In
225 addition, the nutrient conditions vary among seasons in the Yellow Sea (Chen, 2009; Shen,
226 2001; Wang, Wang, & Zhan, 2003). The composition of phytoplankton shifts according to
227 nutrient conditions and the composition of marine bacterial communities may exhibit similar

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228 patterns (Agawin, Duarte, & Agusti, 2000; Liu et al., 2013; Taylor, Cottingham, Billinge, &
229 Cunliffe, 2014). The seasonal difference in crab microbiota could be influenced by
230 differences in nutrient conditions and interactions with other marine organisms.

231 Proteobacteria was a dominant phylum in most crabs (Fig. 3). However, other dominant
232 phyla in crabs differed between spring and autumn. These results were consistent with
233 previous studies indicating that Proteobacteria is the most abundant phylum in the gut and
234 surface of various crabs, regardless of season and location (Givens et al., 2013; Goffredi,
235 Jones, Erhlich, Springer, & Vrijenhoek, 2008; Li et al., 2012; Zhang et al., 2016).
236 *Psychrobacter* was common and was the dominant genus belonging to Proteobacteria in both
237 spring and autumn crabs (Fig. 4). *Psychrobacter* within Proteobacteria can survive at
238 relatively low temperatures and grow at -10 °C to 42 °C (Ayala-del-Rio et al., 2010; Feller,
239 Zekhnini, Lamotte-Brasseur, & Gerday, 1997). Therefore, the relative abundance of
240 *Psychrobacter* was higher in spring than autumn, since it can survive and dominate during the
241 winter (water temperature, 3–4 °C). However, *Roseovarius* within the Alphaproteobacteria
242 was a dominant genus in autumn crabs (Fig. 4B). *Roseovarius* is an important genus in the
243 heterotrophic bacterial community owing to its ability to utilize low-molecular-weight
244 organic compounds, such as dimethylsulfoniopropionate (DMSP) (Buchan, González, &
245 Moran, 2005; Kirkwood, Le Brun, Todd, & Johnston, 2010). Previous studies have shown
246 that DMSP-utilizing ability could be associated with phytoplankton blooms (Buchan et al.,
247 2005; Park et al., 2015; Tan et al., 2015). The dominance of *Roseovarius* in autumn crabs
248 could be related to changes in phytoplankton, since the phytoplankton bloom occurs annually
249 from July to October (characterized by a high water temperature) (NIFS, 2013). *Arcobacter*
250 of Proteobacteria was the dominant genus at A4 in autumn crabs. This genus has been
251 detected in the guts of crabs and in sea environments (Chen et al., 2015; Omoregie et al.,

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252 2008). Therefore, Proteobacteria was the dominant phylum in both spring and autumn, but
253 the dominant genera within Proteobacteria differed between seasons.

254 The relative abundances of Firmicutes in crabs were significantly higher in spring than
255 autumn. In Firmicutes, *Carnobacterium* and *Vagococcus* were dominant genera in spring
256 crabs; these genera have been detected in marine fishes and contribute to cellular immune
257 responses by inhibiting the growth of pathogens (Leisner, Laursen, Prevost, Drider, &
258 Dalgaard, 2007; Román et al., 2012; Sorroza et al., 2012). *Streptococcus* and *Lactococcus*
259 were also dominant genera in the spring. Some species of *Streptococcus* and *Lactococcus*
260 have been recognized as fish pathogens, causing septicemic fish disease, and have resulted in
261 significant losses of fish production as well as spoilage and deterioration of food quality
262 during storage (Fernández-No et al., 2012; Vendrell et al., 2006; Wang et al., 2007). Based on
263 a PCoA plot, the composition of microbiota differed among sampling sites, regardless the
264 distance between sampling sites (Fig. 5). In particular, the dominance of *Lactococcus* in crabs
265 in the spring was associated with a high abundance of potential pathogens at sampling sites
266 (Table 2). Therefore, a high frequency of *Lactococcus* and a low frequency of *Psychrobacter*
267 are potential risk markers according to the microbiota analysis.

268 Although the composition of the crab microbiota can be influenced by the microbiota of
269 their habitat, such as water and sediment, other factors are also important. The composition of
270 microbiota in crab differed among sampling sites, even between sampling sites in relatively
271 close proximity. For example, a shorter distance separated sampling sites 3 and 4 than sites 1
272 and 4 (Fig. 1). However, the compositions of microbiota in crabs at sites 1 and 4 were more
273 similar than those at sites 3 and 4 (Fig. 3 and 4). According to a previous study, the
274 composition of symbiotic bacteria in the gills and guts of crabs was different from the
275 composition of bacteria in the water in which they live (Zhang et al., 2016). Various

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276 additional factors, such as food and interactions with other marine microbes, might influence
277 the microbiota in crabs. Further studies are necessary to analyze the interaction between
278 microbes colonizing crabs and those in surrounding environments.

279 Potential pathogenic bacteria were detected in crabs, regardless of season, but their
280 frequencies were low in most crab samples (Table 2). The relative abundances of *A.*
281 *hydrophila* and *L. garvieae* were higher in spring than autumn. *A. hydrophila* is a marine
282 pathogen that is generally found in the water and seafood, and infection with this bacteria
283 could cause gastroenteritis and septicemia (Daskalov, 2006; Janda & Abbott, 2010). *L.*
284 *garvieae* is considered a fish pathogen, and foodborne outbreaks associated with this bacteria
285 have been reported (Chan et al., 2011; Wang et al., 2007). The relative abundances of
286 potential pathogens were lower in autumn crabs than in spring crabs. Therefore, the potential
287 risk of foodborne illness by the ingestion of crabs is higher in spring than in autumn.
288 However, the presence of potential pathogens was determined by their relative frequencies,
289 and further studies are needed to clarify the pathogenicity of these bacteria.

290

291 **5. Conclusions**

292

293 The microbiota in swimming crabs was more diverse in autumn than in spring, and the
294 composition was related to seasonal factors, such as water temperature and nutrient
295 conditions. *Psychrobacter* within Proteobacteria was the common dominant genus in crabs in
296 both seasons. *Carnobacterium, Lactococcus, Streptococcus,* and *Vagococcus* within
297 Firmicutes were dominant genera in crabs in the spring, whereas *Roseovarius* and *Arcobacter*
298 within Proteobacteria were dominant in the autumn. Potential pathogenic bacteria were
299 detected in crabs, and they were more frequent in spring than autumn, particularly *A.*

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300 *hydrophila* and *L. garvieae*. Additional studies are necessary to clarify the pathogenicity of
301 these bacteria, but these results suggest that the risk of foodborne illness is higher in spring
302 than autumn. This study provides a better understanding of the microbiota in swimming crabs
303 and can facilitate the management and prevention of foodborne illness caused by the
304 consumption of these crabs.

305

306 **Acknowledgements**

307

308 Funding: This research was supported by a grant (14162MFDS972) from the Ministry of
309 Food and Drug Safety, Korea in 2015.

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435 **Figure legends**

436

437 **Fig. 1.** Sampling sites of swimming crabs. Samples were obtained from 6 sites in the spring
438 and autumn. The maximum production areas in South Korea were selected as sampling sites,
439 and the maximum consumption periods in South Korea were selected as sampling seasons.

440

441 **Fig. 2.** Comparisons of bacterial diversity in crabs among sampling seasons and sites. (A)
442 Comparison of diversity between the spring and autumn. Comparison of diversity among
443 sampling sites (B) in the spring and (C) autumn. The p-values were obtained from Mann–
444 Whitney U tests in R. Sample names indicate the sampling season and sampling site (for
445 example, S1: sample collected from site 1 in spring).

446

447 **Fig. 3.** Comparison of the bacterial community among seasons and sites at the phylum level.
448 (A) Comparison of dominant phyla (mean value >1%) between the spring and autumn. (B)
449 Comparison of phyla among sampling sites. The phyla (mean value <1% at each site) were
450 combined and represented as Others. Sample names indicate the sampling season and
451 sampling site (for example, S1: sample collected from site 1 in spring).

452

453 **Fig. 4.** Comparison of the bacterial community at each sampling site at the genus level. (A)
454 Comparison of the genus composition at sampling sites (A) in the spring and (B) autumn. The
455 genera (mean value <1% at each site) were combined and represented as Others. Sample
456 names indicate the sampling season and sampling site (for example, S1: sample collected
457 from site 1 in spring).

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459 **Fig. 5.** Dissimilarities among the bacterial communities in crab samples were examined using
460 a principal coordinate analysis (PCoA) plot. The distance matrix was calculated using the
461 unweighted Fast UniFrac distances. Sample names indicate the sampling season and
462 sampling site (for example, S1: sample collected from site 1 in spring).
463

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Table 1. Summary of diversity indices for crab microbiota.

Samples	# of reads (total)	# of reads (average)[a]	Normalized reads	Average read length (bp)[a]	Observed OTUs[a]	Estimated OTUs (Chao1)[a]	Shannon diversity index[a]	Good's coverage[a]
S1	84,925	16985.00 ± 1781.44	7,900	416.21 ± 20.18	466.40 ± 96.95	819.65 ± 215.40	4.13 ± 0.48	0.97 ± 0.01
S2	56,468	11293.60 ± 2394.45	7,900	370.16 ± 11.01	660.00 ± 139.80	1,290.39 ± 391.98	4.60 ± 0.40	0.96 ± 0.01
S3	68,416	13683.20 ± 2112.65	7,900	405.01 ± 8.04	443.40 ± 79.96	809.76 ± 222.21	3.89 ± 0.33	0.98 ± 0.01
S4	68,896	13779.20 ± 4218.09	7,900	407.46 ± 23.70	490.80 ± 143.13	880.58 ± 276.37	4.18 ± 0.40	0.97 ± 0.01
S5	85,474	17094.80 ± 1918.49	7,900	409.52 ± 16.27	466.60 ± 82.14	820.72 ± 123.22	4.04 ± 0.50	0.97 ± 0.00
S6	71,386	14277.20 ± 1126.71	7,900	375.64 ± 6.57	452.40 ± 49.04	829.35 ± 119.71	3.97 ± 0.34	0.97 ± 0.00
A1	99,149	19829.80 ± 4736.41	7,900	506.65 ± 15.77	1,417.60 ± 270.59	3,309.55 ± 707.73	5.60 ± 0.51	0.89 ± 0.02
A2	89,156	17831.20 ± 2749.24	7,900	507.50 ± 7.37	1,303.80 ± 385.87	2,707.48 ± 823.82	5.40 ± 0.69	0.91 ± 0.03
A3	62,933	12586.60 ± 6978.44	7,900	486.56 ± 16.86	1,395.20 ± 386.98	2,787.14 ± 945.72	6.03 ± 0.32	0.90 ± 0.02
A4	80,987	16197.40 ± 2822.50	7,900	498.39 ± 20.37	1,490.20 ± 182.37	3,303.73 ± 452.85	5.84 ± 0.24	0.89 ± 0.02
A5	85,043	17008.60 ± 2370.55	7,900	492.75 ± 10.83	1,138.20 ± 235.51	2,317.53 ± 518.67	5.44 ± 0.44	0.92 ± 0.02
A6	80,405	16081.00 ± 3469.31	7,900	493.19 ± 9.52	1,699.60 ± 299.10	3,756.13 ± 708.92	6.03 ± 0.45	0.87 ± 0.03

[a] Values indicate means ± S.D (standard deviation) after normalization of reads for 5 crabs from each site.

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Table 2. Proportion of potential pathogens in swimming crabs.

Species	Proportion in microbiota of spring crab[a] (detected samples/5 crabs)[b]						Proportion in microbiota of autumn crab[a] (deteced samples/5 crabs)[b]					
	Site1	Site2	Site3	Site4	Site5	Site6	Site1	Site2	Site3	Site4	Site5	Site6
Aeromonas hydrophila	0.91% (5/5)	2.60% (5/5)	0.05% (4/5)	0.20% (5/5)	0.17% (4/5)	-[c]	-	-	-	-	-	-
Brucella melitensis	-	-	-	-	-	-	-	-	-	-	-	-
Clostridium perfringens	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	0.01% (1/5)	-	-	-	-	-
Lactococcus garvieae	1.30% (5/5)	33.55% (5/5)	0.05% (2/5)	0.20% (3/5)	1.95% (5/5)	27.81 (5/5)	0.02% (1/5)	0.02% (3/5)	-	0.02% (2/5)	-	-
Mycobacterium tuberculosis	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella enterica	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus epidermidis	-	-	-	-	-	-	0.04% (2/5)	-	-	0.24% (2/5)	0.02% (2/5)	0.09% (2/5)
Staphylococcus saprophyticus	0.08% (3/5)	-	-	-	0.20% (3/5)	-	-	-	-	0.01% (2/5)	-	-
Vibrio vulnificus	-	-	-	-	-	-	-	-	-	-	-	0.02% (1/5)

[a] Average proportion of pathogen in microbiota of detected crab samples per site.

[b] The number of detected samples in 5 crabs per site.

[c] -, Not detected.

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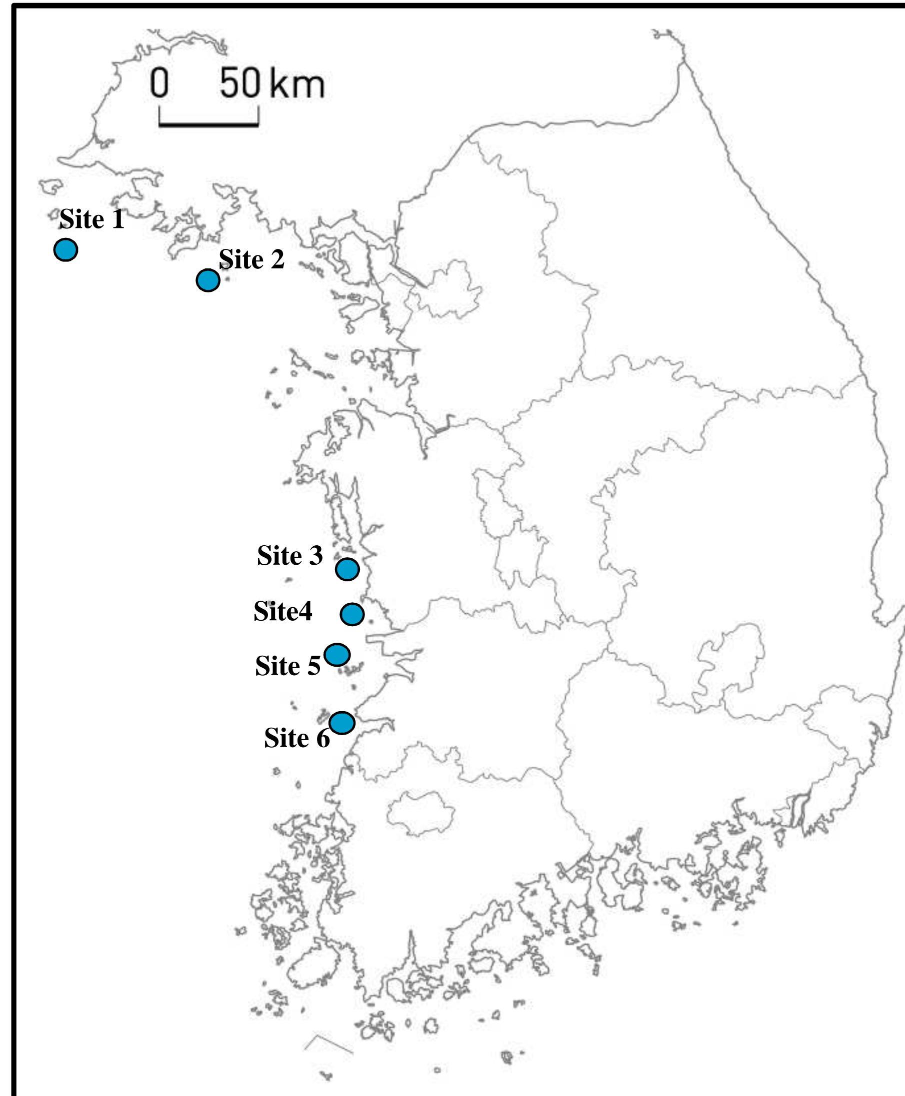
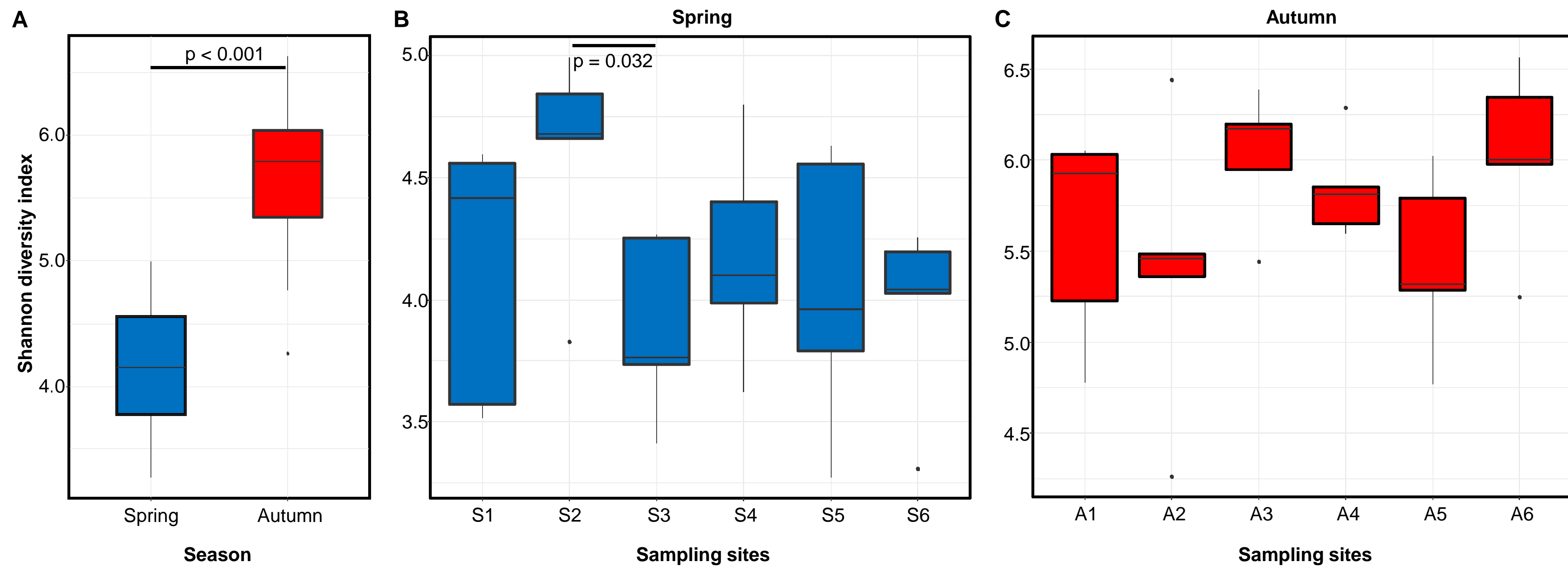
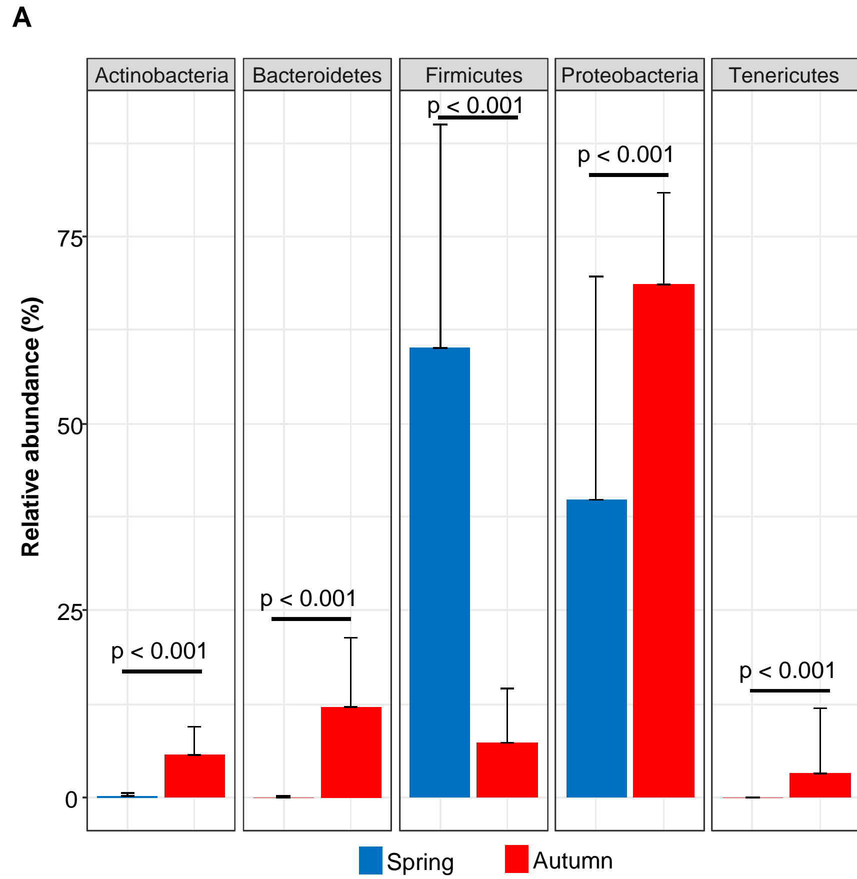
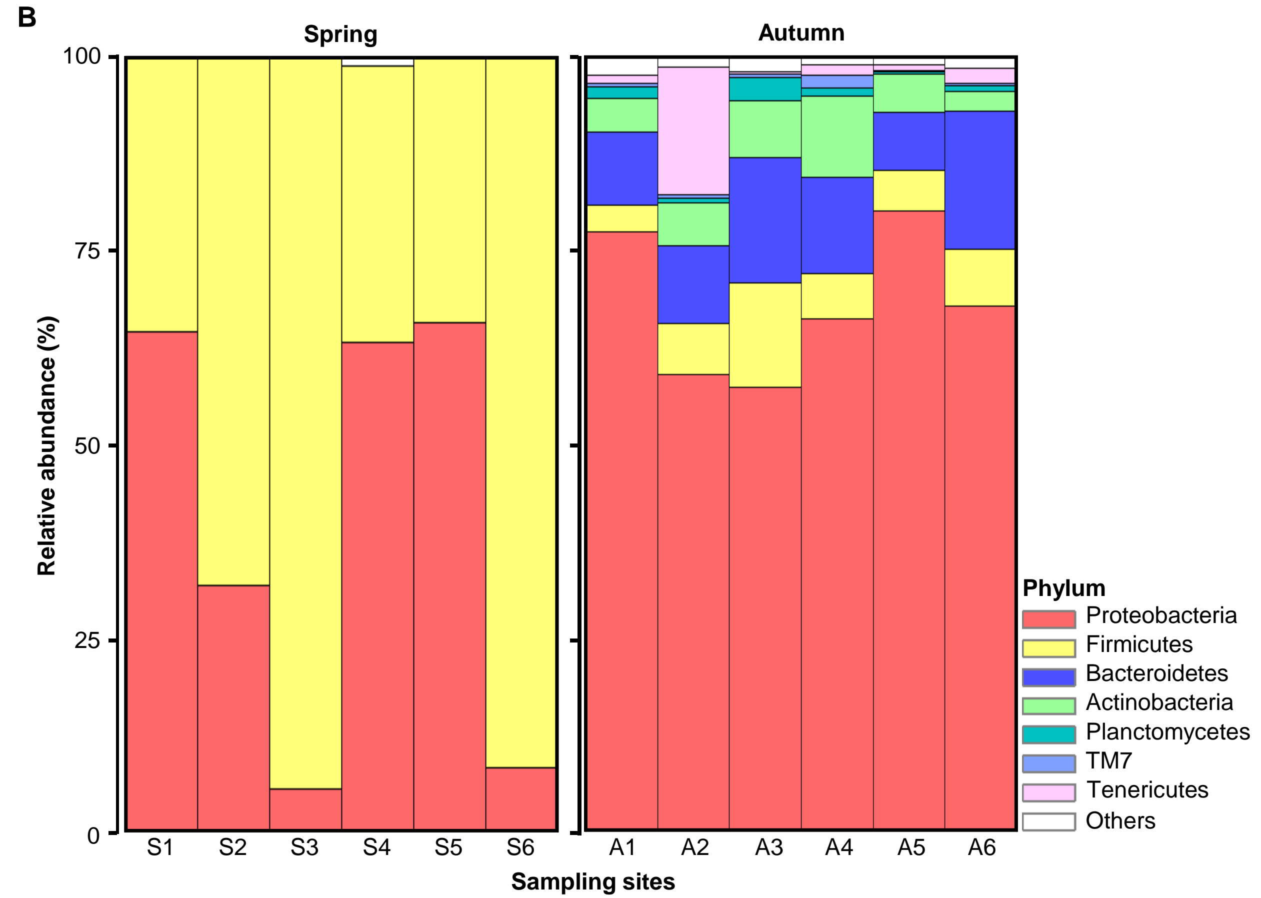

0
50 km
Site 1
Site 2
Site 3
Site4
Site 5
Site 6


A
B
Spring
C
Autumn
Shannon diversity index
p < 0.001
p = 0.032
6.0
5.0
4.0
5.0
4.5
4.0
3.5
6.5
6.0
5.5
5.0
4.5
Spring
Autumn
S1
S2
S3
S4
S5
S6
A1
A2
A3
A4
A5
A6
Season
Sampling sites
Sampling sites


A
Actinobacteria
Bacteroidetes
Firmicutes
Proteobacteria
Tenericutes
p < 0.001
p < 0.001
p < 0.001
p < 0.001
p < 0.001
Relative abundance (%)
0
25
50
75
Spring
Autumn


B
Spring
Autumn
Relative abundance (%)
0
25
50
75
100
S1
S2
S3
S4
S5
S6
A1
A2
A3
A4
A5
A6
Sampling sites
Phylum
Proteobacteria
Firmicutes
Bacteroidetes
Actinobacteria
Planctomycetes
TM7
Tenericutes
Others

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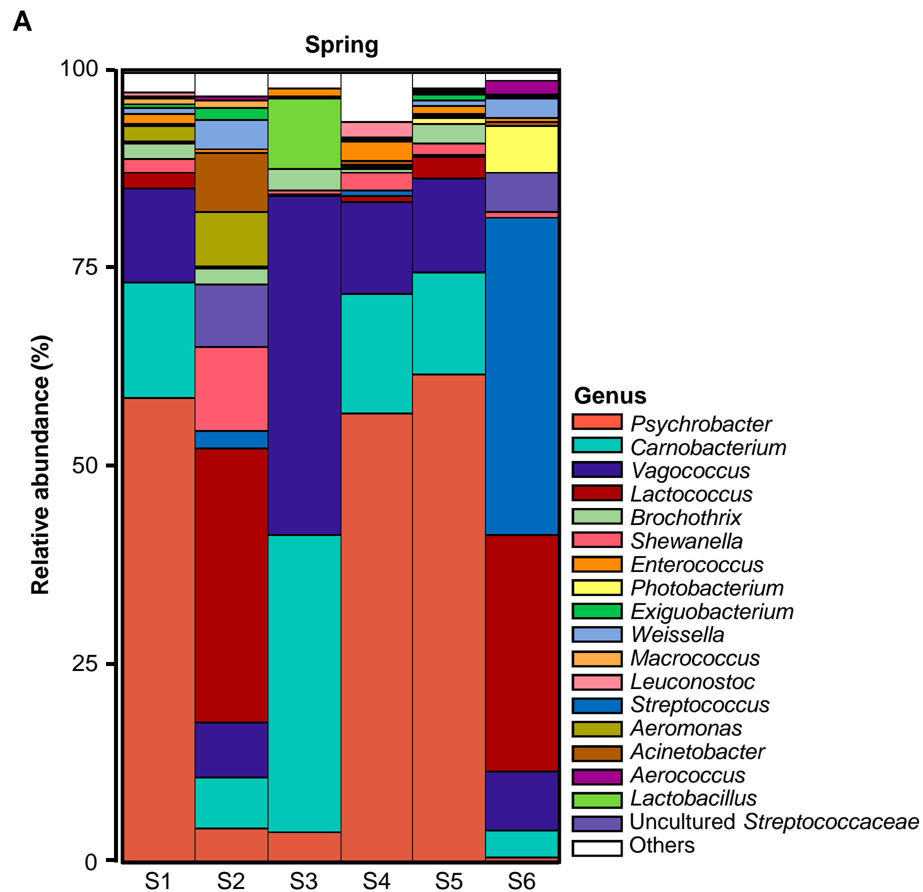

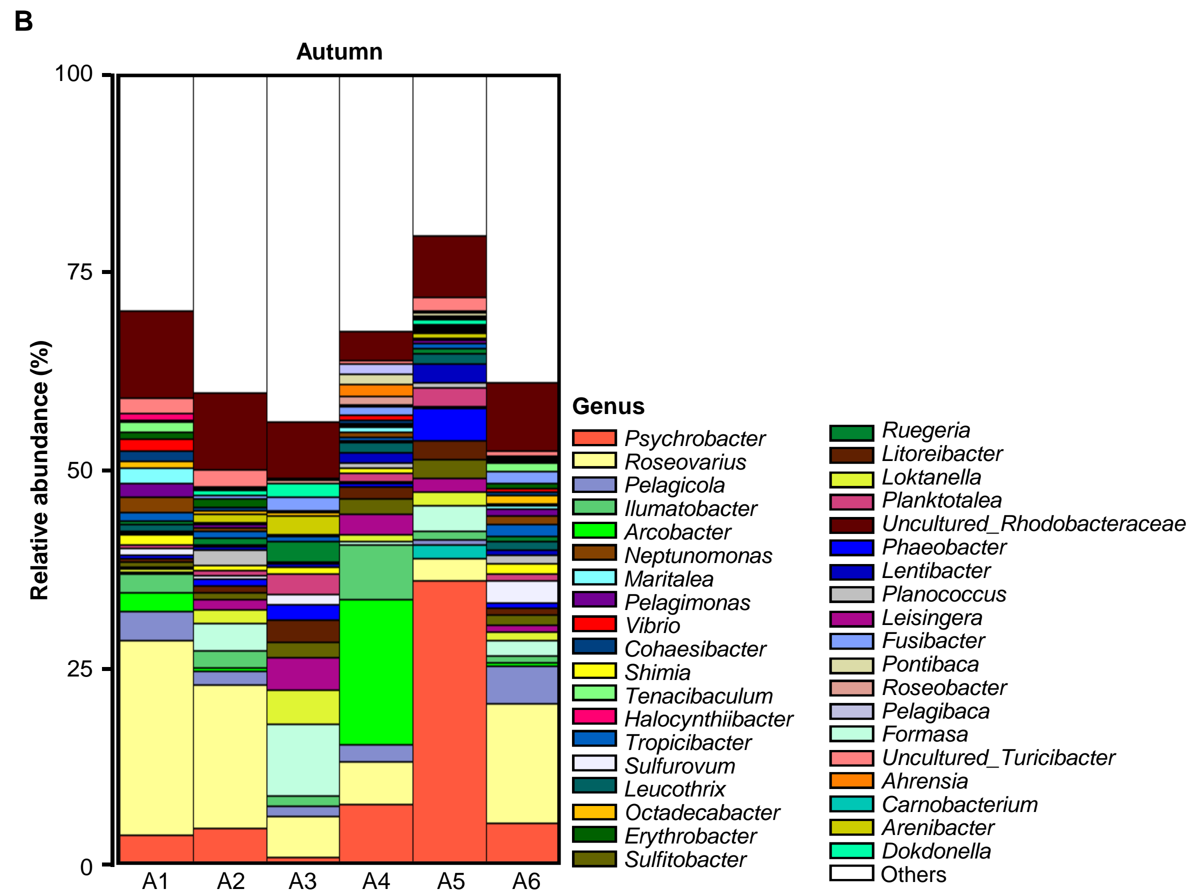

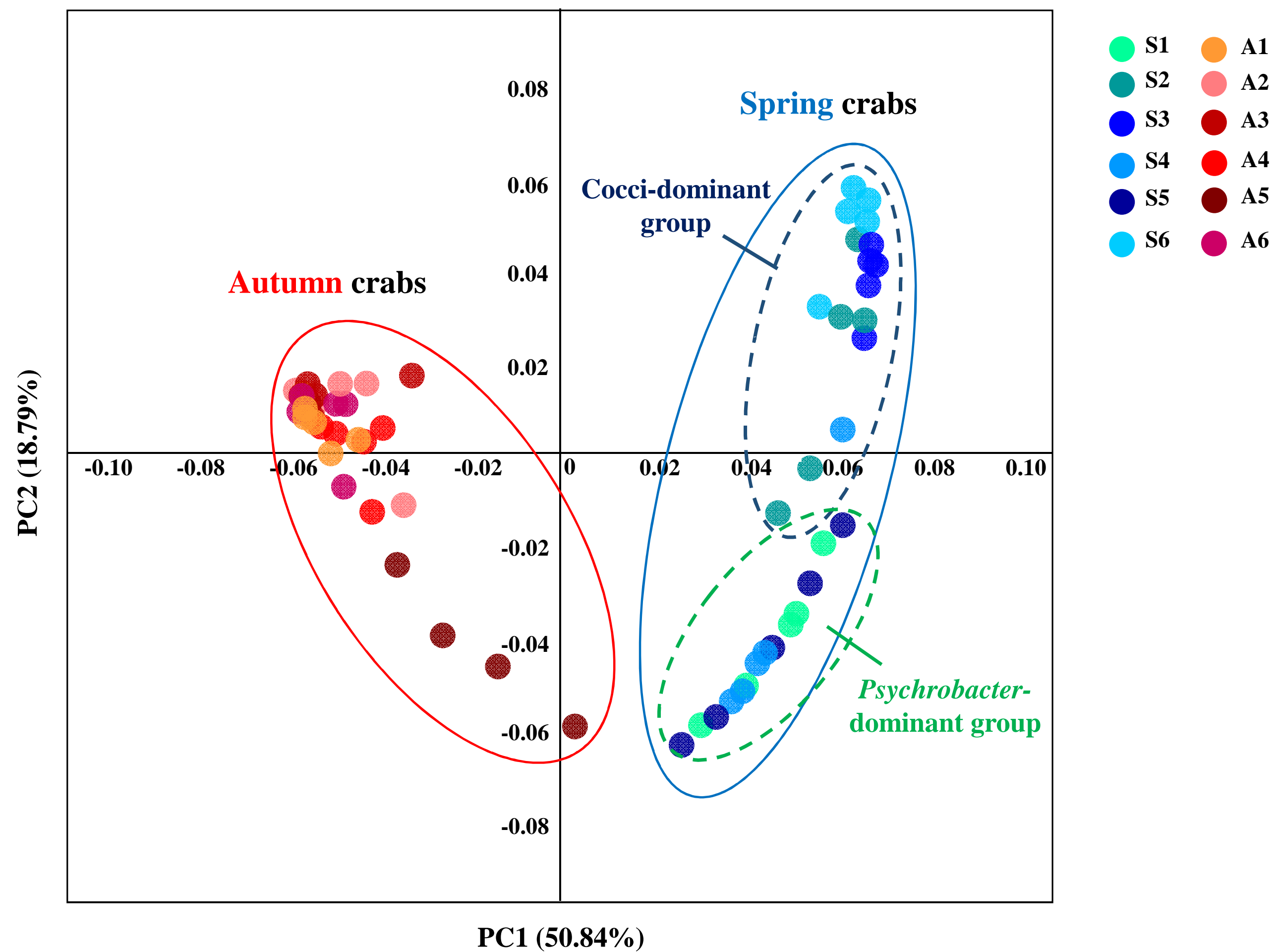
Spring crabs
Autumn crabs
Cocci-dominant group
Psychrobacter-dominant group
S1
S2
S3
S4
S5
S6
A1
A2
A3
A4
A5
A6
0.08
0.06
0.04
0.02
-0.02
-0.04
-0.06
-0.08
-0.10
-0.08
-0.06
-0.04
-0.02
0
0.02
0.04
0.06
0.08
0.10
PC2 (18.79%)
PC1 (50.84%)

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Highlights

- The microbiota in swimming crabs in South Korea was analyzed.
- The diversity and composition of the microbiota differed between spring and autumn.
- The proportion of *Lactococcus* and *Psychrobacter* can be potential risk markers.
- The risk of foodborne illness by crab intake is higher in spring than autumn.
- Microbiota characteristics can inform the management of crab products.

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