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

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ABSTRACT

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

1. Introduction

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

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

Here, we analyzed the microbiota in the whole body of crabs obtained from different sampling sites with high production in Korea using high-throughput sequencing. The microbial compositions of crabs were compared among sampling regions and seasons. In addition, we identified potential pathogens. The study results extend our understanding of food poisoning by crabs and may improve the management of products.

2. Material and methods

2.1. Crab sample collection

A total of 60 swimming crabs (*P. trituberculatus*) were collected

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from 6 sites in the Yellow sea in Korea in May of 2015 (spring) and October of 2014 (autumn) (Fig. 1). Crab consumption is highest in the spring and autumn in South Korea. The areas with the highest production in Korea were chosen as sampling sites (Oh, 2011). Five crabs were randomly collected at each site and were immediately transported in an icebox to the laboratory.

2.2. Metagenomic DNA extraction

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

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.

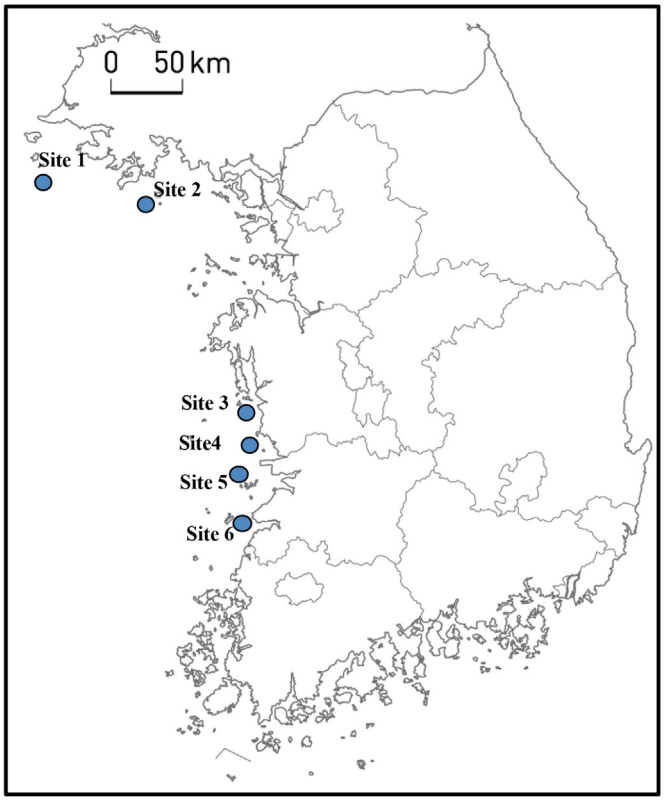

Fig. 1. Sampling sites of swimming crabs. Samples were obtained from 6 sites in the spring and autumn. The maximum production areas in South Korea were selected as sampling sites, and the maximum consumption periods in South Korea were selected as sampling seasons.

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

2.5. Statistical analysis

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

3. Results

3.1. Comparison of microbial diversity among crab samples

A total of 933,238 reads (average 14,519 reads per spring sample and 16,589 reads per autumn sample) were analyzed after quality filtering (Table 1). The diversity indices for samples were compared after read numbers were normalized by random subsampling. The average Good's coverage was 0.94 ± 0.04. The Shannon diversity index was higher for crabs collected in autumn than in spring (Fig. 2; $p < 0.001$). The microbiota for crabs exhibited the highest diversity at site 2 and the lowest diversity at site 3 in the spring. Microbial diversity was highest for in crabs from site 6 and lowest for crabs at site 2 in the autumn. A significant difference in diversity between sampling sites within the same sampling season was only detected between sites 2 and 3 in the spring ($p = 0.032$). There were no significant differences in microbial diversity among sampling sites in the autumn ($p > 0.05$).

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	16,985.00 ± 1781.44	7900	416.21 ± 20.18	466.40 ± 96.95	819.65 ± 215.40	4.13 ± 0.48	0.97 ± 0.01
S2	56,468	11,293.60 ± 2394.45	7900	370.16 ± 11.01	660.00 ± 139.80	1290.39 ± 391.98	4.60 ± 0.40	0.96 ± 0.01
S3	68,416	13,683.20 ± 2112.65	7900	405.01 ± 8.04	443.40 ± 79.96	809.76 ± 222.21	3.89 ± 0.33	0.98 ± 0.01
S4	68,896	13,779.20 ± 4218.09	7900	407.46 ± 23.70	490.80 ± 143.13	880.58 ± 276.37	4.18 ± 0.40	0.97 ± 0.01
S5	85,474	17,094.80 ± 1918.49	7900	409.52 ± 16.27	466.60 ± 82.14	820.72 ± 123.22	4.04 ± 0.50	0.97 ± 0.00
S6	71,386	14,277.20 ± 1126.71	7900	375.64 ± 6.57	452.40 ± 49.04	829.35 ± 119.71	3.97 ± 0.34	0.97 ± 0.00
A1	99,149	19,829.80 ± 4736.41	7900	506.65 ± 15.77	1417.60 ± 270.59	3309.55 ± 707.73	5.60 ± 0.51	0.89 ± 0.02
A2	89,156	17,831.20 ± 2749.24	7900	507.50 ± 7.37	1303.80 ± 385.87	2707.48 ± 823.82	5.40 ± 0.69	0.91 ± 0.03
A3	62,933	12,586.60 ± 6978.44	7900	486.56 ± 16.86	1395.20 ± 386.98	2787.14 ± 945.72	6.03 ± 0.32	0.90 ± 0.02
A4	80,987	16,197.40 ± 2822.50	7900	498.39 ± 20.37	1490.20 ± 182.37	3303.73 ± 452.85	5.84 ± 0.24	0.89 ± 0.02
A5	85,043	17,008.60 ± 2370.55	7900	492.75 ± 10.83	1138.20 ± 235.51	2317.53 ± 518.67	5.44 ± 0.44	0.92 ± 0.02
A6	80,405	16,081.00 ± 3469.31	7900	493.19 ± 9.52	1699.60 ± 299.10	3756.13 ± 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.

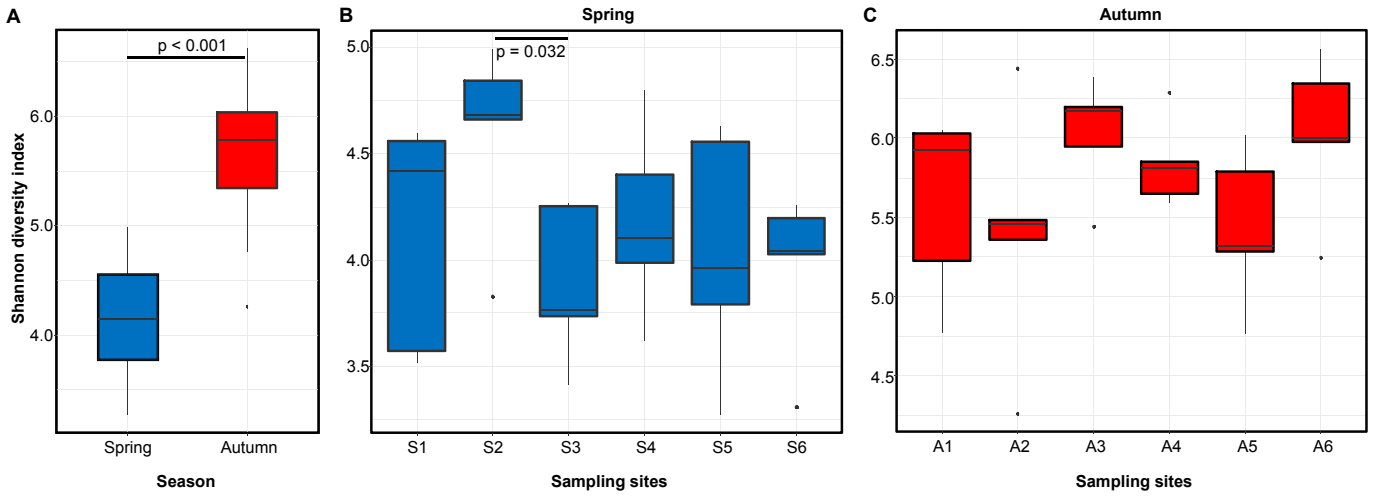

Fig. 2. Comparisons of bacterial diversity in crabs among sampling seasons and sites. (A) Comparison of diversity between the spring and autumn. Comparison of diversity among sampling sites (B) in the spring and (C) autumn. The p-values were obtained from Mann–Whitney U tests in R. Sample names indicate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

3.2. Comparison of the microbial composition in crabs from different sampling seasons and sites

The composition of the crab microbiota differed significantly between spring and autumn at the phylum level ($p < 0.001$; Fig. 3). Proteobacteria and Firmicutes were dominant in the microbiota of all crabs in the spring, whereas diverse phyla were detected in the microbiota of crabs in the autumn. The relative abundance of Firmicutes was higher in spring samples than in autumn samples (Fig. 3A; $p < 0.001$). The relative abundances of other dominant phyla, including Proteobacteria, Bacteroidetes, Actinobacteria, and Tenericutes, were higher in autumn samples than in spring samples ($p < 0.001$).

Firmicutes was the dominant phylum in crabs from sites S3 (mean, 94.6%), S6 (91.79%), and S2 (68.21%), whereas Proteobacteria was dominant at S5 (65.67%), S1 (64.55%), and S4 (63.08%) in the spring (Fig. 3B). Various microbial phyla were detected in crabs in the autumn. Proteobacteria was the dominant phylum (57.22%–80.22%) at all sampling sites in the autumn. The highest proportion of Proteobacteria was detected in crabs from site A5, and the lowest proportion was detected at site A3 ($p < 0.05$). Bacteroidetes was the next most dominant phylum at all sampling sites, except A2 (7.5%–17.73%). Although the proportion of Bacteroidetes was highest at site A6, the difference between sampling sites was not significant ($p > 0.05$). Tenericutes was the second most abundant phylum at A2 (16.56%). The third most dominant phylum was variable among sampling sites in the autumn.

The crab microbiota was further examined at the genus level. *Psychrobacter* was the dominant genus in samples dominated by Proteobacteria (61.80% in S5, 58.96% in S1, and 56.94% in S4) in the spring ($p < 0.01$ for the difference between Proteobacteria-dominant sites and Firmicutes-dominant sites; Fig. 4A). Various genera were dominant in Firmicutes-dominant samples. *Lactococcus* (34.46%) and *Shewanella* (10.67%) were the dominant genera at S2 ($p < 0.01$ in comparison with other sites, except S6); *Carnobacterium* (37.69%) and *Vagococcus* (42.86%) were dominant genera at S3 ($p < 0.05$ in comparison with other sites); *Lactococcus* (29.89%) and *Streptococcus* (40.11%) were dominant genera at S6 ($p < 0.05$ in comparison with other sites, except S1 and S4). The composition of genera was more diverse in autumn than in spring. However, Proteobacteria was dominant in all crab samples in the autumn, and *Psychrobacter* was the dominant genus only at site A5 (35.71%). *Roseovarius* within Alphaproteobacteria was the dominant genus in the autumn; its relative abundance was highest at site A1 (24.67%). *Arcobacter* (18.59%) was the dominant genus at site A4 ($p < 0.05$ compared with other sites). In addition, uncultured bacteria were more common in autumn (20.17%–40.61%) than in spring (0.15%–8.47%). The dominant genera in the crab microbiota (mean

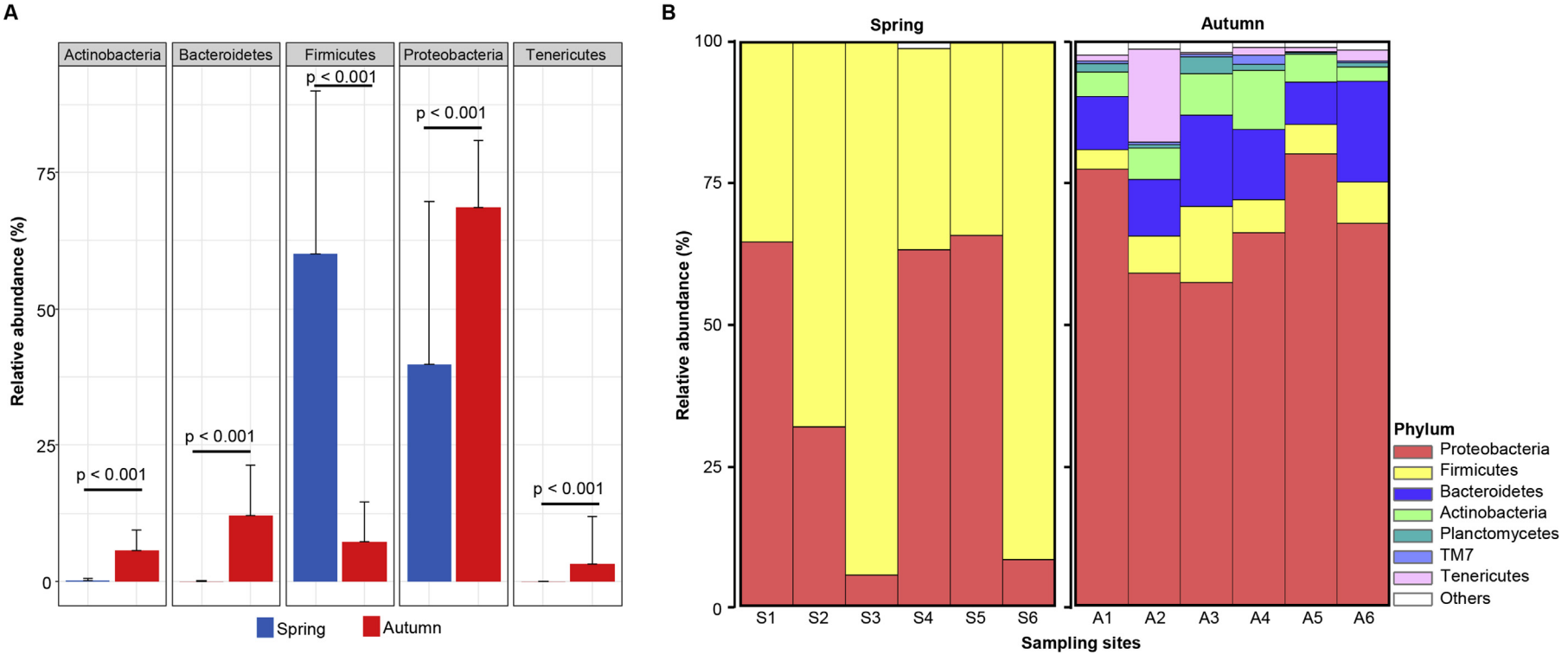

Fig. 3. Comparison of the bacterial community among seasons and sites at the phylum level. (A) Comparison of dominant phyla (mean value > 1%) between the spring and autumn. (B) Comparison of phyla among sampling sites. The phyla (mean value < 1% at each site) were combined and represented as Others.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

proportion >1% at each site) differed significantly between the spring and autumn ($p < 0.01$; Supplementary Fig. S2). The relative abundances of *Psychrobacter*, *Carnobacterium*, *Vagococcus*, *Lactococcus, Streptococcus*, and *Shewanella* were higher in spring than autumn, whereas those of *Roseovarius*, *Pelagicola*, *Arcobacter*, *Formosa*, *Ilumatobacter*, uncultured *Clostridiales*, uncultured *Rhodobacteraceae*, and uncultured *Lumbricoplasmataceae* were higher in autumn than spring.

The difference in crab microbiota between sampling seasons was also investigated by a principal coordinates analysis (PCoA) based on unweighted UniFrac distances (Fig. 5). The crab microbiota was clearly separated between spring and autumn samples. The microbiota was more similar among sampling sites for autumn crabs than spring crabs. The microbiota of crabs from A5 was different from those of crabs from other sites in the autumn. However, the spring crabs were subdivided into two groups, a cocci-dominated group (S2, 24, and S5) and a *Psychrobacter*-dominated group (S1, S3, and S6).

3.3. Potential pathogens in swimming crabs

The frequencies of 11 species related to foodborne illness were analyzed to identify potential pathogens in the swimming crab (Daskalov, 2006; Iwamoto, Ayers, Mahon, & Swerdlow, 2010; Scallan et al., 2011) (Table 2). The sum of the frequencies of foodborne pathogens was higher in spring (average 11.48± 14.75%) than in autumn (0.03± 0.04%), and the presence of pathogenic bacteria differed depending on the season. The pathogenic species *Aeromonas hydrophila* was detected only in the spring, whereas *Escherichia coli* and *Staphylococcus epidermidis* were detected in the autumn. The average proportion of *Lactococcus garvieae,* a potential foodborne pathogen, was higher in spring (10.81%) than in autumn (0.02%).

The detection of pathogenic bacteria also differed among sampling sites within the same season. In the spring, the proportion of pathogenic bacteria was relatively high at site 2 (36.15%) and site 6 (27.81%) compared to other sites (0.1%–2.32%) owing to the abundance of *L. garvieae* at these sites. Although *A. hydrophila* was detected in all spring crab samples except site 6, it was more abundant at site 2 (2.60%) than at other sites. In the autumn, the relative abundances of foodborne pathogens were lower at site 2 (0.02%), site 3 (not detected), and site 5 (0.02%) than at other sites (0.07%–0.27%).

4. Discussion

In this study, the swimming crab microbiota was analyzed and compared among seasons and sites in the Yellow sea in South Korea. The diversity and composition of the crab microbiota differed significantly between spring and autumn (Figs. 2 and 3). The differences in microbiota among sampling sites were relatively small compared to the differences between seasons. These results indicated that the crab microbiota is strongly influenced by season, and accordingly might be related to differences in water temperature. The temperature of the Yellow Sea is highest in August (24.8–25.1 °C) and lowest in February (3.0–4.1 °C), while the salinity is fairly steady throughout the year (29.3–31.5‰) (KOOFS, 2015). The microbiota in spring crabs was influenced by the relatively low temperature during the winter, whereas that in autumn crabs was influenced by the higher temperature during the summer (Moisander, Sexton, & Daley, 2015; Pinto, Schroeder, Lunn, Sloan, & Raskin, 2014). Therefore, the microbial diversity was higher in autumn crabs than in spring crabs owing to the higher temperature, which promotes the growth of diverse microbes. In addition, the nutrient conditions vary among seasons in the Yellow Sea (Chen, 2009; Shen, 2001; Wang, Wang, & Zhan, 2003). The composition of phytoplankton shifts according to nutrient conditions and the composition of marine bacterial communities may exhibit similar patterns (Agawin, Duarte, & Agusti, 2000; Liu et al., 2013; Taylor, Cottingham, Billinge, & Cunliffe, 2014). The seasonal difference in crab microbiota could be influenced by differences in nutrient conditions and interactions with other marine organisms.

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

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

Although the composition of the crab microbiota can be influenced by the microbiota of their habitat, such as water and sediment, other factors are also important. The composition of microbiota in crab differed among sampling sites, even between sampling sites in relatively close proximity. For example, a shorter distance separated sampling sites 3 and 4 than sites 1 and 4 (Fig. 1).

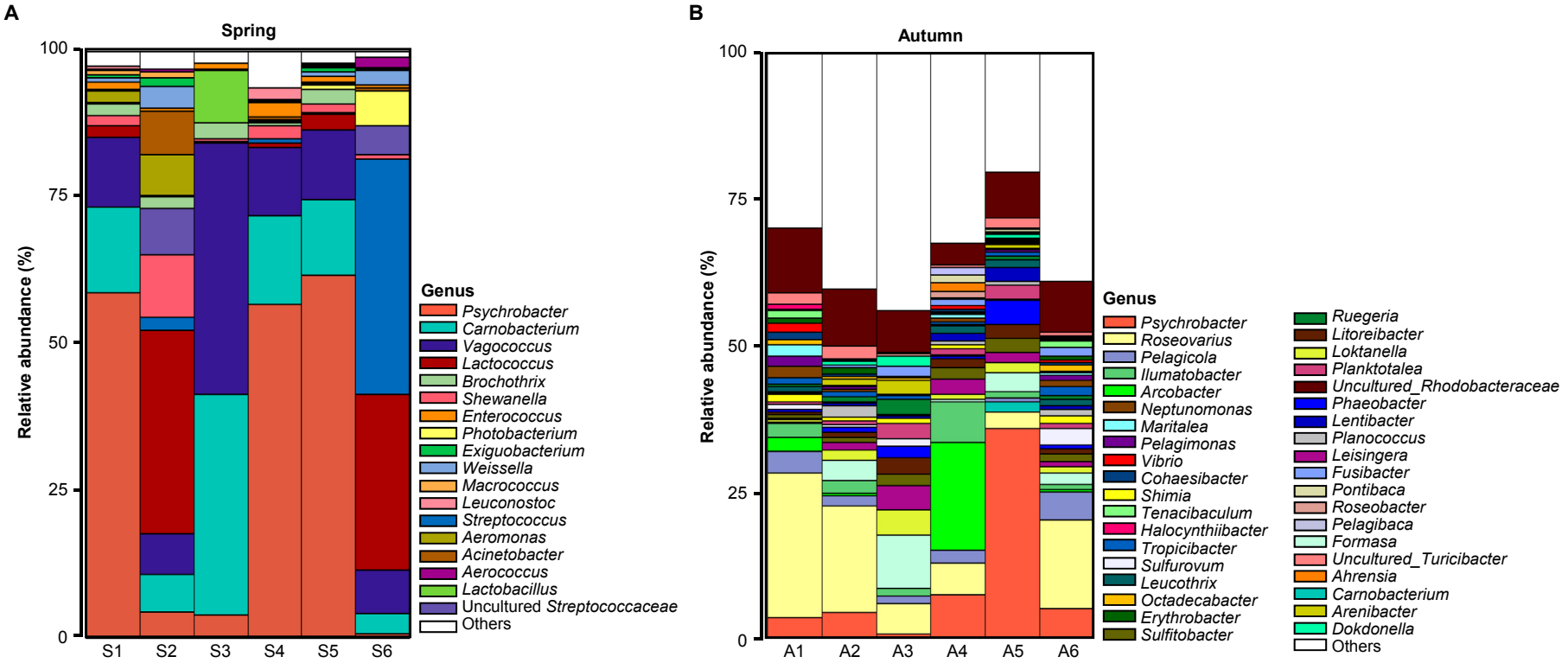

Fig. 4. Comparison of the bacterial community at each sampling site at the genus level. (A) Comparison of the genus composition at sampling sites (A) in the spring and (B) autumn. The genera (mean value < 1% at each site) were combined and represented as Others. Sample names indicate the sampling season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

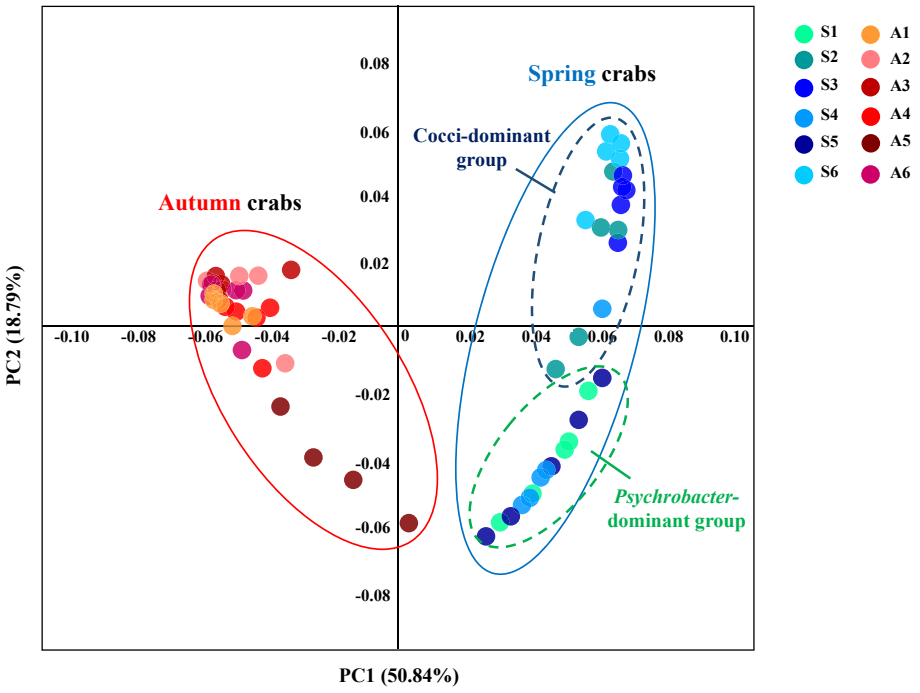

Fig. 5. Dissimilarities among the bacterial communities in crab samples were examined using a principal coordinate analysis (PCoA) plot. The distance matrix was calculated using the unweighted Fast UniFrac distances. Sample names indicate the sampling season and sampling site (for example, S1: sample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

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] (detected 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.

However, the compositions of microbiota in crabs at sites 1 and 4 were more similar than those at sites 3 and 4 (Figs. 3 and 4). According to a previous study, the composition of symbiotic bacteria in the gills and guts of crabs was different from the composition of bacteria in the water in which they live (Zhang et al., 2016). Various additional factors, such as food and interactions with other marine microbes, might influence the microbiota in crabs. Further studies are necessary to analyze the interaction between microbes colonizing crabs and those in surrounding environments.

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

5. Conclusions

The microbiota in swimming crabs was more diverse in autumn than in spring, and the composition was related to seasonal factors, such as water temperature and nutrient conditions. *Psychrobacter* within Proteobacteria was the common dominant genus in crabs in both seasons. *Carnobacterium, Lactococcus, Streptococcus,* and

Vagococcus within Firmicutes were dominant genera in crabs in the spring, whereas *Roseovarius* and *Arcobacter* within Proteobacteria were dominant in the autumn. Potential pathogenic bacteria were detected in crabs, and they were more frequent in spring than autumn, particularly *A. hydrophila* and *L. garvieae*. Additional studies are necessary to clarify the pathogenicity of these bacteria, but these results suggest that the risk of foodborne illness is higher in spring than autumn. This study provides a better understanding of the microbiota in swimming crabs and can facilitate the management and prevention of foodborne illness caused by the consumption of these crabs.

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Appendix ASupplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.08.043.

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