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Microbial community of retail marinated raw crab: Comprehensive analysis via culture-dependent methods and high-throughput sequencing

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ABSTRACT

Marinated raw crab, a popular pickled seafood product in Korea, is consumed without heat treatment and is therefore considered a potential reservoir for foodborne pathogens. Despite its popularity, microbiological investigations of commercial marinated raw crab remain limited. This study aimed to characterize bacterial profiles using culture-dependent methods and 16S rRNA amplicon sequencing. Crab marinated in soy sauce (n = 120) and spicy sauce (n = 120) were collected across four seasons. Average aerobic plate counts and coliform counts were 4.73 log and 1.41 log CFU/g for soy sauce and 5.31 log and 1.80 log CFU/g for spicy sauce, respectively. 16S rRNA sequencing identified abundant lactic acid bacteria and spoilage-causing or problematic bacteria. While *Psychrobacter*, *Vibrio*, and *Carnobacterium* were dominant in soy sauce, *Psychrobacter*, *Photobacterium*, and *Weissella* prevailed in spicy sauce. Although many bacterial genera were shared, microbial community structure differed by sauce type. *Bacillus*, *Weissella*, and *Staphylococcus* were more abundant in spicy sauce, potentially due to red pepper powder, a key ingredient. Additionally, seasonal analysis revealed significant bacterial count variations for soy sauce samples and higher spoilage organism levels in warmer months for both sauce types, emphasizing the need for proper temperature control. This study characterizes microbial communities in commercial marinated raw crab and provides foundational data to enhance microbiological safety.

1. Introduction

In 2022, the global production of aquatic animals reached a new world record of 185 million tons (live weight equivalent), an increase of 4 % from 2020 (Food and Agriculture Organization of the United Nations [FAO], 2024). Globally, apparent consumption per capita of aquatic animal foods increased at an average rate of about 1.4 percent per year, from 9.1 kg in 1961 to 20.6 kg in 2021 (FAO, 2024). In response to the increasing demand for seafood, South Korea's total marinated raw crab exports amounted to 923 tons in volume and USD 5 million in value, reflecting its growing popularity overseas (Korea Fishery Trade Association, 2024).

Marinated raw crab, a highly regarded traditional Korean delicacy renowned for its distinctive flavor, is categorized as a fermented seafood product (Ministry of Food and Drug Safety [MFDS], 2024). There are two main types of marinated raw crab: crab marinated in soy sauce and crab marinated in spicy sauce, both typically prepared using the same species of raw crab (*Portunus trituberculatus*) (Y. Chen et al., 2023). The main distinction lies in the primary marinating ingredient: soy sauce is usually prepared by fermenting soybeans in brine, whereas red pepper powder is the key flavoring component of spicy sauce. To manufacture crab marinated in soy sauce, soy sauce is boiled first with garlic, onion, ginger, pepper, and apple, then cooled and added to raw crab to marinate at temperatures of 5 or 22 °C for 5–6 days (Cho et al., 2016). To manufacture crab marinated in spicy sauce, the spicy sauce made with red pepper powder, soy sauce, starch syrup, garlic, and ginger is mixed directly with raw crab and typically consumed after mixing or a short marination period of 1–2 days.

Marination can suppress the growth of pathogens because of external stresses, such as high salt content, low water activity, low pH, and the presence of preservatives (Rhoades et al., 2013). Therefore, consumers often considered marinated raw crabs as microbiologically safe.

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However, microorganisms may persist or even thrive under the inhibitory conditions, potentially affecting safety and quality. As a ready-to-eat (RTE) food that undergoes no heat treatment, marinated raw crab can pose a risk if pathogens are present. Crabs are known to occasionally carry bacterial pathogens such as *Vibrio parahaemolyticus*, *Salmonella* spp., *Escherichia coli*, *Staphylococcus epidermidis*, and *Listeria monocytogenes* that can cause foodborne illnesses (Ahmed et al., 2018; Matulkova et al., 2013; Pagadala et al., 2012; Talpur et al., 2011; Ukwo, 2024). In 2018, an outbreak of *V. parahaemolyticus* infections linked to crab meat resulted in 26 cases of illness and 9 hospitalizations in the United States (Seelman et al., 2023). In 2011, the United Kingdom reported 8 cases of *E. coli* O157:H7 infections attributed to crab consumption (Matulkova et al., 2013). According to the Consumer Injury Surveillance System (CISS), there were 185 reported incidents related to marinated raw crab over a period of 3.5 years (from January 2015 to June 2018) in Korea. Many of these cases involved symptoms of foodborne illness, including abdominal pain, vomiting, and diarrhea (CISS, 2018).

To address these safety concerns and understand the microbiological ecology of marinated raw crab, comprehensive empirical data on the associated microbiota is required. Methodologies for investigating microbial communities can be either culture-dependent or culture-independent. Culture-based methods offer advantages such as the quantification of bacterial load, the detection of only viable bacteria, the ability to isolate specific foodborne pathogens, and the provision of valuable phenotypic information (Vaz-Moreira et al., 2011; B. Wang et al., 2017). However, culture-dependent methods may be insufficient to examine the bacterial communities. The underestimation of total bacterial counts often occurs due to the undetected presence of viable but non-culturable (VBNC) bacteria (Besnard et al., 2000; Foddai & Grant, 2020; Li et al., 2014; Nocker et al., 2007). Additionally, microbial communities are dynamic in both space and time, with differences in replication rates allowing fast-growing species to efficiently outcompete others (Giraffa & Neviani, 2001; Nocker et al., 2007). Advancements in DNA-based techniques, such as next-generation sequencing (NGS), also known as high-throughput sequencing (HTS), help to overcome these limitations, enabling the comprehensive detection of bacteria, even those uncultivable (P. Chen et al., 2016; M. Jang et al., 2021; S. A. Kim et al., 2017). Researchers are achieving a more comprehensive understanding of microbial communities in food by simultaneously implementing culture-dependent and culture-independent approaches, thereby leveraging the strengths of each method (H. Chen et al., 2008; Iacumin et al., 2009; Nielsen et al., 2007; Temmerman et al., 2004).

While some studies have analyzed the microbiomes of raw crab (Deng et al., 2019; Givens et al., 2013; S. Kim et al., 2017; Qin et al., 2024; Sun et al., 2018; N. Zhang et al., 2017), very few have examined those of marinated raw crab. No research has been conducted on large-scale retail samples with different sauce types and from different sampling seasons. The present study combines culture-dependent methods with culture-independent 16S rRNA sequencing to evaluate microbial communities across a large sample of retail marinated raw crab products, aiming to provide a comprehensive understanding of their microbial ecology. To the best of our knowledge, this is the first study to investigate microbial variations in commercially distributed marinated raw crab by sauce type. Additionally, to evaluate the impact of seasonal variations on the bacterial communities, samples were collected seasonally.

2. Materials and methods

2.1. Sample collection

Between March 2022 and March 2023, crab marinated in soy sauce ($n = 120$) and crab marinated in spicy sauce ($n = 120$) were collected from various retail stores in Seoul and Sejong, South Korea. A more detailed description of the collected samples is shown in supplementary data. Fig. 1 presents representative images of marinated raw crab products investigated in the current study. Each sample was systematically categorized based on the season of its collection, as detailed in Table 1. The sampling season was divided into four categories according to the seasonal definition used by the Korea Meteorological Administration: spring (March to May), summer (June to August), fall (September to November), and winter (December to February). The shelf life of the samples was typically 4 days under refrigerated storage and 1 month under frozen storage conditions, with the sampling season closely aligned with the manufacturing season. To maintain the original temperature, all specimens were transported in ice bags and delivered to the laboratory within 2 h.

2.2. Culture-dependent microbial analysis

2.2.1. Quantitative analysis

Quantitative analysis was performed on aerobic plate counts (APC), coliforms, and *E. coli* according to the scientific methodologies outlined in the Food Code (MFDS, 2024). For each marinated raw crab, 25 g of the sample was aseptically taken and placed into a sterile 3M™ filter sample bag (3M, St. Paul, MN, USA) containing 225 mL of sterile 0.85 % saline and homogenized at 230 rpm for 2 min in a stomacher (Circulator 400, Seward, Worthing, UK). The homogenized sample (1 mL) was subjected to 10-fold serial dilutions in 9 mL of sterile 0.85 % saline. From each dilution, 1 mL was plated in duplicate onto 3M™ Petrifilm Aerobic Count Plate (AC) and 3M™ Petrifilm *E. coli*/Coliform Count Plates (EC/CC) (3M). The plates were incubated at 37 °C for 48 and 24 h, respectively. After incubation, red colonies on AC petrifilm were counted as APCs. Blue colonies associated with gas bubbles on EC/CC petrifilm were identified as *E. coli*, and red and blue colonies associated with gas production were classified as total coliforms. The average of the duplicate colony counts is calculated as a logarithmic value.

2.2.2. Qualitative analysis

Qualitative analysis was conducted to identify the presence of *E. coli*, *L. monocytogenes*, and *Salmonella enterica* serovar Typhimurium, major foodborne pathogens, using selective/differential culture media and Sanger sequencing. Each 25 g of the edible part of the sample was aseptically collected and homogenized in a 3M™ filter sample bag with 225 mL of Tryptic Soy Broth (Difco, Becton Dickinson Co., Sparks, MD, USA) at 230 rpm for 2 min using a stomacher. The homogenized sample bags were incubated at 37 °C for 24 h. Every selective/differential medium was performed in duplicate.

To isolate *E. coli*, a loopful of the enriched cultures was streaked onto CHROMagar™ *E. coli* and incubated at 37 °C for 24 h. Blue colonies were then transferred to BBL™ Eosin Methylene Blue Agar (Difco) and incubated under the same conditions. Blue-black colonies with a green metallic sheen were subsequently streaked onto Tryptic Soy Agar (TSA, Difco) and incubated again at 37 °C for 24 h in preparation for identification via Sanger sequencing.

For the detection of *L. monocytogenes*, a loopful of the enriched cultures was streaked onto CHROMagar™ *Listeria* and incubated at 37 °C for 24 h. Colonies exhibiting a blue color with a white halo were then transferred to *Listeria* Selective Agar Base (Oxford Formulation) (Oxoid, Hampshire, UK) and incubated for 24 h at 37 °C. Presumptive brown-colored colonies were streaked onto TSA and incubated once more at 37 °C for 24 h, also for Sanger sequencing.

To identify *S.* Typhimurium, each enrichment was inoculated onto CHROMagar™ *Salmonella* and incubated at 37 °C for 24 h. The mauve colonies were transferred to Xylose Lysine Desoxycholate Agar (Difco) and cultured at 37 °C for 24 h. Red colonies with black cores were streaked onto TSA and incubated under the same conditions. To identify the strains, DNA was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions, and the DNA sequence of all isolated colonies was analyzed by Sanger sequencing at BIONICS Co., Ltd. (South Korea).



Fig. 1. Representative images of crab marinated in soy sauce and crab marinated in spicy sauce.

Table 1
The number of samples collected for each season.

Sample type	Season				Total
	Spring	Summer	Fall	Winter	
Crab marinated in soy sauce	30	30	30	30	120
Crab marinated in spicy sauce	30	30	30	30	120

2.3. Culture-independent 16S rRNA amplicon sequencing analysis of microbial profiles

2.3.1. DNA extraction

To concentrate the sample prior to DNA extraction, 40 mL of the sample homogenized in sterile 0.85 % saline was centrifuged at 12,000 rpm for 20 min at 4 °C in a LaboGene 1696R centrifuge (LaboGene, Daejeon, Korea). After discarding the supernatant, leaving a volume of approximately 5 mL, the pellet was resuspended in the remaining supernatant and homogenized again. The concentrated samples were stored at −20 °C until further analysis. DNA was extracted with the DNeasy PowerSoil Pro Kit (QIAGEN) according to the manufacturer's guidelines. The purity and concentration of the extracted DNA were verified by following the nucleic acid quantitation protocol using a microplate reader (Tecan Spark, Tecan Trading AG, Zurich, Switzerland).

2.3.2. Library preparation

Library preparation was conducted according to previous studies (M. Jang et al., 2021; S. A. Kim et al., 2017; S. Y. Kim et al., 2022; Kozich et al., 2013). PCR was conducted using dual-index primers to amplify the V4 region of the 16S rRNA gene from the DNA samples (crab marinated in soy sauce: $n = 120$, crab marinated in spicy sauce: $n = 120$). Gel electrophoresis was conducted to verify successful amplification, and results were inspected using the Gel Doc XR c150 system (Bio-Rad, Hercules, CA, USA). Crab marinated in soy sauce ($n = 6$) and crab marinated in spicy sauce ($n = 9$) were excluded from the next steps due to unsuccessful amplification caused by low DNA concentrations. Successfully amplified PCR products ($n = 225$) were normalized using the SequalPrep™ Normalization Plate Kit (Applied Biosystems, Waltham, MA, USA) following the manufacturer's instructions. For quality check, the concentrations of the normalized products were further verified using a Qubit 4.0 (Thermo Fisher Scientific Inc., Waltham, MA, USA). Five microliters from each product were combined in a LoBind microcentrifuge tube (Eppendorf, Hamburg, Germany) for library preparation, and pooled library quality was examined by qPCR analysis (QuantStudio™ 1, Applied Biosystems) using the KAPA Library Quantification Kit (KK4824, Kapa Biosystems, Wilmington, NC, USA).

2.3.3. 16S rRNA gene sequencing on illumina MiSeq platform

The double-stranded DNA of the pooled library was denatured by combining it with PhiX control v3 (Illumina, San Diego, CA, USA) and 0.2N NaOH. The library and PhiX control v3 were diluted to a final concentration of 6 pM using HT1 buffer, and then 480 μL of 6 pM library and 120 μL of 6 pM PhiX control were mixed. The mixture was loaded into a MiSeq® Reagent cartridge v2 (500 cycles; Illumina) for sequencing. The sequencing progress was monitored in real-time through the Illumina BaseSpace® website (https://basespace.illumina.com/dashboard), and sequencing reads were downloaded in the FASTQ file format.

2.3.4. Processing sequencing data through bioinformatics

Demultiplexed Read 1 and Read 2 sequences were subjected to further processing and analysis. The sequence files were analyzed using Quantitative Insights Into Microbial Ecology 2 (QIIME 2, version 2023.2), and the Divisive Amplicon Denoising Algorithm 2 (DADA2) package was used to trim sequencing data to remove low-quality reads, PhiX, and chimeric sequences for reliable results. Processed sequence data (crab marinated in soy sauce: $n = 104$, crab marinated in spicy sauce: $n = 103$) were aligned, and the taxonomic classification of amplicon sequence variants (ASVs) was performed based on the SILVA 16S rRNA gene database. QIIME 2 and MicrobiomeAnalyst (version 2.0) (Chong et al., 2020) were used to assess the alpha diversity (Chao1 and Shannon indices) of the bacterial community. Linear discriminant analysis effect size (LEfSe) analysis was conducted to determine the most discriminant taxa based on the relative abundance (Segata et al., 2011a). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2, version 2.4.1) was used to profile microbial functional potentials based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Douglas et al., 2020).

2.4. Statistical analysis and data visualization

The bacterial counts (log CFU/g) were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc Honestly Significant Difference (HSD) test, implemented in R software (version 4.4.1). A p-value of <0.05 was considered statistically significant. Taxonomic relative abundance data from QIIME 2 analysis were visualized using Microsoft Excel (version 2308; Microsoft, Redmond, WA, USA). Alpha diversity was evaluated with Chao1 and Shannon indices in MicrobiomeAnalyst, and statistical analysis was conducted by pairwise comparisons with the Mann–Whitney test. Differences between microbial communities among samples were evaluated using Permutational Multivariate Analysis of Variance (PERMANOVA). The Statistical Analysis of Metagenomic Profiles (STAMP, version 2.1.3) software was used for plotting PICRUSt-derived data to ascertain significant differences in the abundance of metabolic pathways (Welch's t-test, 0.95 confidence interval [CI], two-sided, Storey's false discovery rate [FDR] $q < 0.05$) between groups (Parks et al., 2014).

2.5. Data records

The 16S rRNA raw sequence data for the samples analyzed in this study were deposited into the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) under the accession number PRJNA1190929.

3. Results and discussion

3.1. Analysis based on sample sauce type

3.1.1. Bacterial population by culture-dependent methods

Fig. 2 shows the APC and CC of crab marinated in soy sauce ($n = 120$) and crab marinated in spicy sauce ($n = 120$). The APC of crab marinated in soy sauce ranged from 2.41 to 7.81 log CFU/g (average 4.73 ± 1.13 log CFU/g), and the APC of crab marinated in spicy sauce ranged from 3.17 to 6.82 log CFU/g (average 5.31 ± 0.64 log CFU/g). The corresponding CC levels averaged 1.41 ± 1.13 and 1.80 ± 1.15 log CFU/g, respectively. The maximum detected CC was 4.25 log CFU/g in crab marinated in soy sauce and 4.98 log CFU/g in crab marinated in spicy sauce.

The APC and CC of crab marinated in spicy sauce were significantly higher than those of crab marinated in soy sauce ($p < 0.05$). These results may be related to the primary ingredient in the sauce, as red pepper powder is frequently associated with high levels of microbial contamination. Previous studies have reported that the average APC of red pepper powder is approximately 4.00–7.12 log CFU/g (Kyung et al., 2019; Woldemariam et al., 2021). Additionally, the relatively lower bacterial counts observed in soy sauce samples may be attributed to the boiling process of the marinade mixture.

In the qualitative analysis, *E. coli* was detected in two samples of crab marinated in spicy sauce. *L. monocytogenes* and *S.* Typhimurium were not detected in any of the marinated raw crab samples. Based on the 1:10 sample dilution and 1 mL plating volume, the theoretical detection limit of the quantitative pathogen analysis was 5 CFU/g. In contrast, the qualitative pathogen analysis included an enrichment step, which theoretically allows for the detection of even a single viable cell. However, false-negative results may still have occurred due to the presence of bacteria in VBNC state or the uneven distribution of pathogens in the sample.

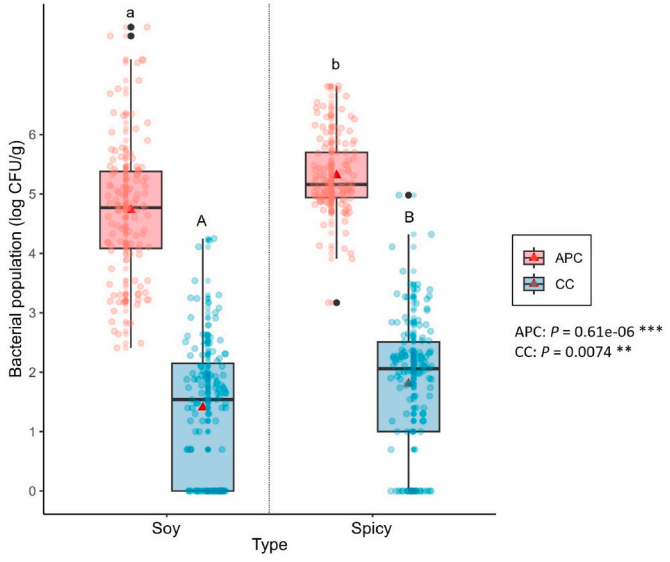


Fig. 2. Aerobic plate counts (APC; pink) and coliform counts (CC; blue) of crab marinated in soy sauce ($n = 120$) and crab marinated in spicy sauce ($n = 120$). The red triangles denote the mean values; each point indicates an individual sample. Different letters above the whiskers indicate significant differences within each count type ($p < 0.05$).

3.1.2. Culture-independent microbial community analysis through 16S rRNA sequencing

Of the initial 240 samples, 23 were excluded during quality assessment, and 217 samples proceeded to high-throughput sequencing (HTS), including 107 soy sauce-marinated and 110 spicy sauce-marinated crab samples. One additional sample from the soy sauce group was removed during the denoising step, leaving 216 samples. To ensure adequate sequencing depth, 10 samples with fewer than 10,000 reads were excluded, resulting in a final dataset of 206 high-quality samples used for microbiome analysis. The sequencing process was monitored in real time using the Illumina BaseSpace® platform, and quality control metrics confirmed successful sequencing, with 93.08 % of base calls above Q30, a low error rate (1.71 %), and a passing filter percentage of 87.90 %.

The taxonomic abundance profile at the genus level is shown in Fig. 3. A total of 1287 genera were identified, with only the top 20 displayed for each of the soy sauce and spicy sauce samples in Fig. 3A. Overall, *Psychrobacter* (24.72 %), *Vibrio* (12.76 %), and *Carnobacterium* (12.63 %) were dominant in crab marinated in soy sauce. The microbiological composition of crab marinated in spicy sauce exhibited distinctions from crab marinated in soy sauce, with several dominant genera, including *Psychrobacter* (27.63 %), *Photobacterium* (6.65 %), *Weissella* (6.06 %), and *Bacillus* (6.04 %).

Fig. 3B focuses on the relative abundance of lactic acid bacteria (LAB) and spoilage-causing or problematic bacteria within the samples. LAB are a group of Gram-positive bacteria that utilize carbohydrates to produce lactic acid as a main metabolite and include food-related genera: *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Lactiplantibacillus*, *Lacticaseibacillus*, *Lactosphaera*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Vagococcus*, and *Weissella* (da Silva Sabo et al., 2014; Y. Wang et al., 2021; Zheng et al., 2020). *Carnobacterium* (12.63 %) and *Leuconostoc* (3.86 %) were the predominant genera in crab marinated in soy sauce; conversely, *Weissella* (6.06 %) and *Leuconostoc* (5.09 %) showed higher relative abundance in crab marinated in spicy sauce.

The high prevalence of *Carnobacterium* in crab marinated in soy sauce aligns with a previous study that identified this genus as a dominant component in raw crab microbiomes (S. Kim et al., 2017), suggesting that the *Carnobacterium* found in soy sauce samples likely originated from the raw crab itself. Its resilience to freezing, thawing, and low temperatures supports its frequent occurrence in various food products, including seafood (Leisner et al., 2007). The dominance of *Weissella* in the crab marinated in spicy sauce appears to be influenced by the main ingredient, red pepper powder. A previous study reported that members of the genus *Weissella* were present in low abundance in both cabbage kimchi and radish kimchi without red pepper powder yet were significantly more abundant in kimchi varieties containing red pepper powder (S. Jeong et al., 2013). These differences suggest possible variations in the microbial environment influenced by the different sauce ingredients.

For the spoilage-causing or problematic bacteria, *Psychrobacter* was the most abundant spoilage-causing bacterium (24.72 % in soy sauce samples and 27.63 % in spicy sauce samples), followed by *Photobacterium* (5.84 % in soy sauce samples and 6.65 % in spicy sauce samples) and *Pseudomonas* (2.46 % in soy sauce samples and 3.69 % in spicy sauce samples). These bacteria thrive at low temperatures and are commonly associated with the spoilage of refrigerated or frozen seafood products. Crustaceans, including crabs, shrimp, and lobsters, contain a higher concentration of free amino acids than fish, providing an easily accessible substrate for bacterial development. Spoilage results in the production of amines, sulfides, alcohols, aldehydes, ketones, and organic acids, leading to off-odors and off-flavors (Bekaert et al., 2015).

Previous studies have found that the spoilage profiles of fish and crustaceans are relevant to their microbial composition (Broekaert et al., 2013; Jääskeläinen et al., 2019; Lou et al., 2021). Microorganisms with

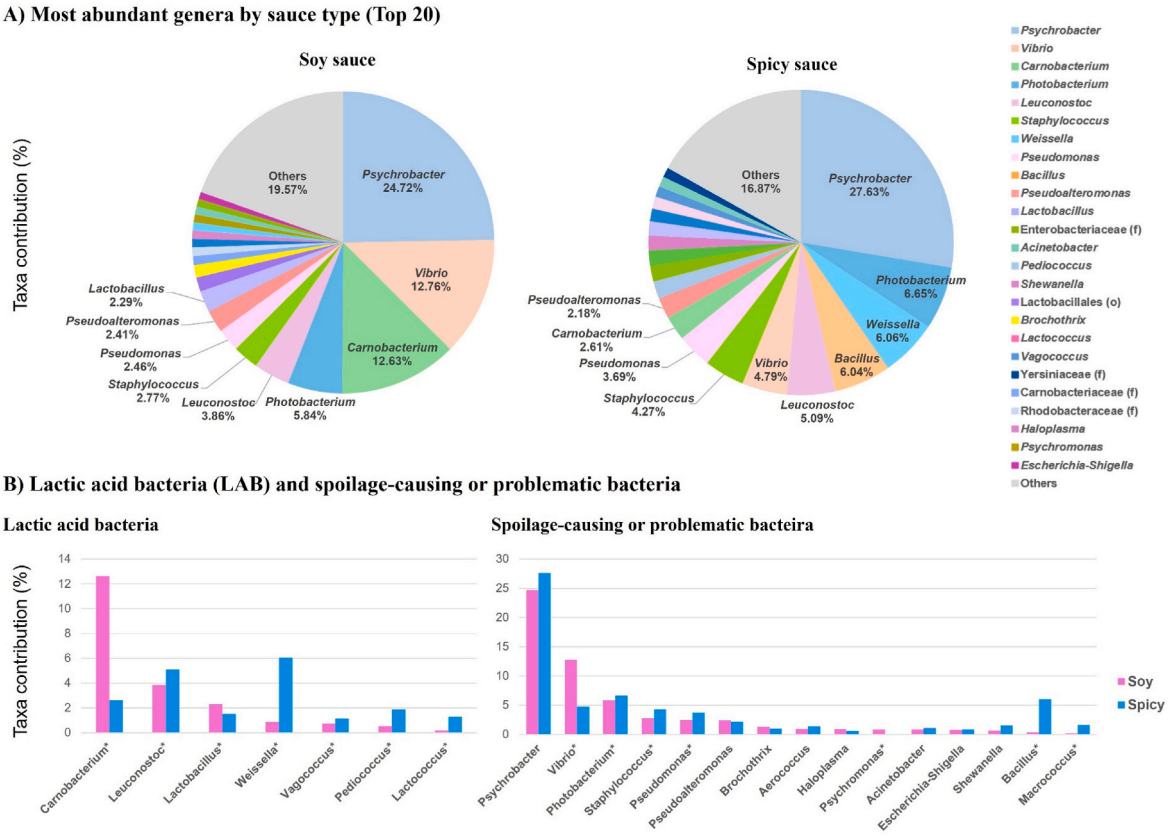


Fig. 3. The relative abundance of soy sauce-marinated raw crab ($n = 103$) and spicy sauce-marinated raw crab ($n = 103$) at the genus level. (A) shows the 20 most prevalent genera, the size of each segment in the pie chart represents the relative abundance of the corresponding taxa within each sample type. (B) categorizes these genera into lactic acid bacteria (LAB) and problematic bacteria. Statistical significance is denoted by an asterisk (*).

spoilage potential (qualitative ability to produce off-odors) and spoilage activity (quantitative ability to produce metabolites) are referred to as specific spoilage organisms (SSOs) (Boziaris & Parlapani, 2017). *Shewanella* spp., *Photobacterium phosphoreum*, and *Pseudomonas* spp. are SSOs that produce volatiles like trimethylamine, ammonium, and hydrogen sulfide (H_2S), indicators in the evaluation of the spoilage level (Boziaris & Parlapani, 2017; Dalgaard, 1995; Tryfinopoulou et al., 2002). The genus *Shewanella* is frequently reported to be the main spoilage microorganism in aerobically stored chilled fish (Boziaris & Parlapani, 2017). The proportion of *Shewanella* was observed to be 0.64 % in the soy sauce samples and 1.54 % in the spicy sauce samples. Although its relative abundance was relatively low, *Shewanella* is known to be associated with spoilage in seafood products. *Shewanella* spp. can grow rapidly at low temperatures (4–8 °C) and exhibit proteolytic activity even under refrigeration conditions, leading to potential changes in texture and sensory characteristics of seafood products (Serio et al., 2014).

Among the problematic bacteria, *Vibrio* and *Staphylococcus* were prevalent in both sauce types (*Vibrio* at 12.76 % in soy sauce samples and 4.79 % in spicy sauce samples; *Staphylococcus* at 2.77 % in soy sauce and 4.27 % in spicy sauce). Both *Vibrio* and *Staphylococcus* are commonly found in marine organisms (Rahmati & Labbe, 2008; Stratev et al., 2021; Çanak & Timur, 2020). *Bacillus* was observed at a higher abundance in spicy sauce samples (6.04 %) compared to soy sauce samples, likely because *Bacillus* is frequently detected in red pepper powder (Choo et al., 2007; A. Jeong et al., 2010; S. K. Kim et al., 2009; Oh et al., 2012).

The antagonistic effects of LAB against problematic bacteria have been examined in various aquatic organisms, revealing that LAB can inhibit the colonization of problematic bacteria in the digestive tract and synthesize antimicrobial peptides called bacteriocins (Evangelista et al., 2023; Gatesoupe, 1999; Leisner et al., 2007; Ringø et al., 2005; Yang et al., 2019). In Fig. 3B, this antagonistic effect is evident, as the crab marinated in soy sauce, with a higher proportion of LAB (21.18 %), shows lower levels of spoilage-causing or problematic bacteria (54.73 %) compared to the crab marinated in spicy sauce, in which the abundance of these bacteria was 19.52 % and 60.61 %, respectively. In addition to the antagonistic effects of LAB, microbial communities interact through a variety of antagonistic and symbiotic relationships. These interactions, including competition for nutrients, nutritional interdependencies, production of inhibitory compounds such as antimicrobial compounds or volatile organic compounds (VOCs), or mutualistic metabolic exchanges, contribute to shaping the overall microbial composition. For example, *Pseudomonas* can also produce VOCs that act as infochemicals within microbial groups. Similarly, certain *Photobacterium* strains produce antimicrobial compounds such as holomycin, which exhibit inhibitory activity against *Enterococcus*, *Escherichia*, *Staphylococcus*, *Vibrio* and *Pseudoalteromonas*. (Hassani, Durán, & Hacquard, 2018; Lau et al., 2022).

The Venn diagram in Fig. 4A illustrates the shared and unique genera between the crabs marinated in different sauces. A total of 1287 genera were identified across all samples, with 257 (20.0 %) unique genera found in the crab marinated in soy sauce and 272 (21.1 %) unique genera found in the crab marinated in spicy sauce. Despite using the same raw material, differences in taxonomic composition were observed based on the type of sauce. The 758 (58.9 %) shared genera, including *Psychrobacter*, *Vibrio*, *Carnobacterium*, *Photobacterium*, and *Staphylococcus*, are commonly found in diverse aquatic environments, such as seawater, freshwater, and marine sediments (Parlapani, 2021; J. Zhang et al., 2019).

Fig. 4B presents the alpha diversity indices (Chao1 and Shannon) for two types of samples. The Chao1 index estimates community richness by predicting the actual number of taxa, whereas the Shannon index is

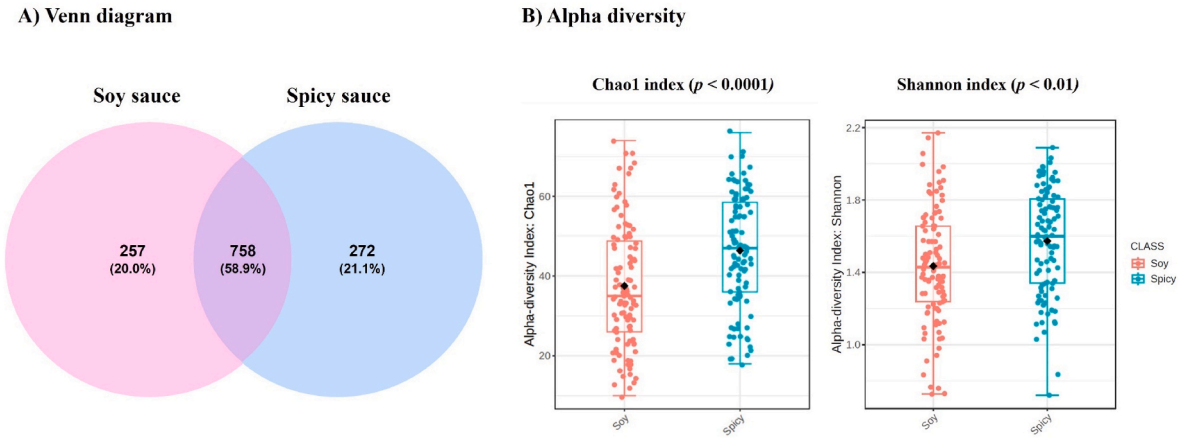


Fig. 4. (**A**) Venn diagram illustrates the shared and unique taxonomic classifications of amplicon sequence variants (ASVs) between crab marinated in soy sauce and crab marinated in spicy sauce. (**B**) Alpha diversity expressed by Chao1 ($p < 0.001$) and Shannon ($p < 0.01$) indices according to the type of samples.

more influenced by the evenness of taxon abundance (Chernov et al., 2015). Both the Chao1 and Shannon indices indicate higher values for the crab marinated in spicy sauce than for the crab marinated in soy sauce, suggesting a relatively more diverse and evenly distributed microbial community in the spicy sauce samples. Fig. 4A shows that the crab marinated in spicy sauce (21.1 %) had more unique ASVs than the crab marinated in soy sauce (20.0 %). The alpha diversity analysis in Fig. 4B further supports this observation.

Fig. 5 explores the differences in microbial composition between the crab marinated in soy sauce and the crab marinated in spicy sauce. In Fig. 5A, the LEfSe analysis demonstrates differentially abundant taxa between the types of samples (LDA score > 2.0). For example, *Carnobacterium* and *Psychromonas* were more abundant in crab marinated in soy sauce, and *Bacillus*, *Weissella,* and *Staphylococcus* were more prevalent in crab marinated in spicy sauce. The LEfSe analysis was performed to identify taxa that contribute the most to the microbial differences between the two sample groups by combining statistical significance with effect size (Chang et al., 2022; Segata et al., 2011b). Fig. 5B shows the results of univariate analysis, which was performed following the LEfSe analysis to independently validate the statistical significance of the top four genera with the highest LDA scores. The univariate analysis tests individual taxa to confirm group-specific abundance differences (Aldirawi & Morales, 2023). These analyses provide important insights into the microbial variations between the two sample groups, with the identified taxa potentially serving as distinguishing features that reflect the effects of soy and spicy sauce marinades.

Fig. 6 presents the microbial functional prediction analysis results derived from PICRUSt2, which integrates open-source tools to predict genome functions associated with sampled 16S rRNA gene sequences. During this process, ASVs are placed into a reference phylogenetic tree, forming the basis for functional predictions (Douglas et al., 2020). The results suggest that pathways related to lipopolysaccharide (LPS) biosynthesis and the *V. cholerae* pathogenic cycle may be more abundant in soy sauce samples, and fatty acid biosynthesis and D-alanine metabolism are predicted to be relatively more represented in spicy sauce samples. LPS is crucial for the survival of Gram-negative bacteria, supporting their structural integrity and pathogenic functions (Dong et al., 2017; X. Wang & Quinn, 2010). Pathways linked to the *V. cholerae* pathogenic cycle facilitate the bacterium's transition from environmental reservoirs to host environments (Tsou et al., 2008). Peptidoglycan, a major component of the cell wall of Gram-positive bacteria, forms a thick protective layer that enhances structural stability (Pasquina-Lemonche et al., 2020). D-alanine is essential for peptidoglycan assembly and cross-linking, providing additional strength to the

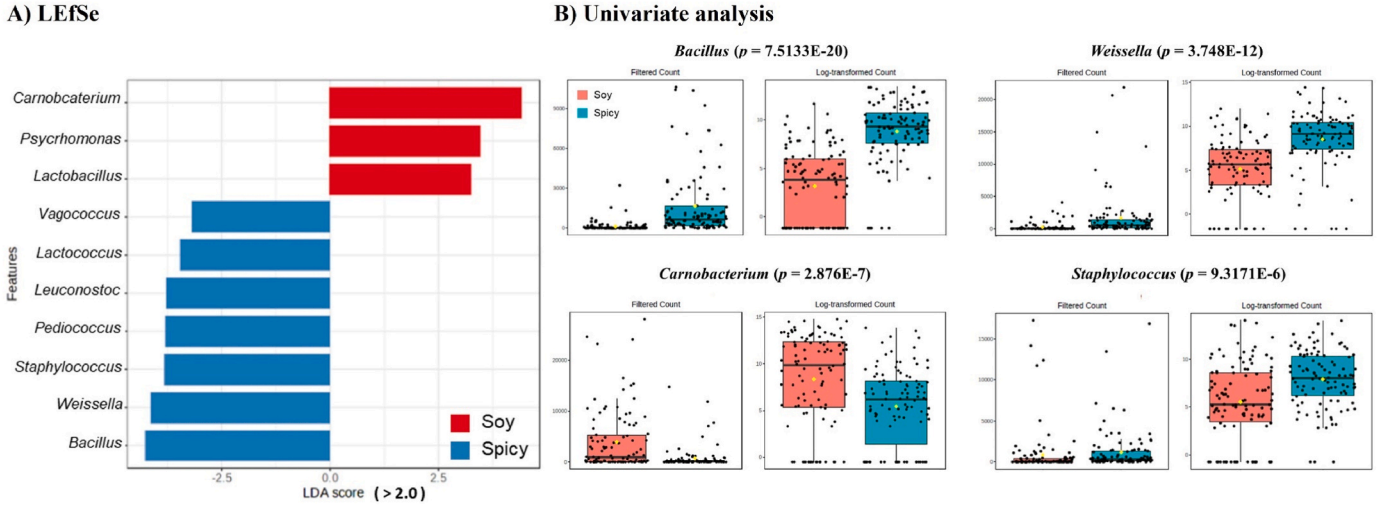


Fig. 5. (**A**) Linear discriminant analysis effect size (LEfSe) revealing taxa differentiating between soy and spicy sacue sample, with LDA scores indicating significant discriminative features and taxa with an LDA score greater than 2.0 and a *p*-value < 0.05 being considered significantly different between the two groups. (LEfSe analysis performed with LDA score 2.0 and $p < 0.05$ as the significance threshold). (**B**) Univariate analysis focusing on microbial genera with the largest differences between soy and spicy sacue samples, such as *Bacillus*, *Weissella*, *Carnobacterium*, and *Staphylococcus* ($p < 0.05$).

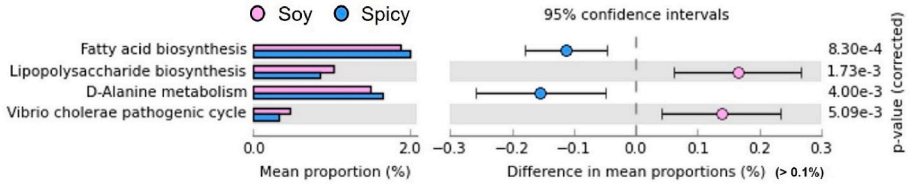


Fig. 6. Functional prediction of marinated raw crab using PICRUSt2 and annotated with KEGG pathways. Extended error bar plot showing significant variations in biosynthetic and metabolic processes for each group (Welch's test, $p < 0.05$).

cell wall (Walsh, 1989). Additionally, fatty acid biosynthesis supports cell wall integrity and membrane fluidity by forming phospholipids as primary membrane components, thus enhancing structural resilience (De Carvalho & Caramujo, 2018). However, as PICRUSt2 relies heavily on reference genome databases and ancestral state reconstruction, the predicted functional profiles may not fully reflect the actual microbial functions present in the samples. Therefore, these results should be interpreted with caution, and future validation using metagenomic or metatranscriptomic approaches is warranted (Matchado et al., 2024).

3.2. Analysis based on sampling season

3.2.1. Bacterial population by culture-dependent methods

Table 2 summarizes the seasonal means of bacterial counts, while Fig. 7 illustrates these variations using box plots to show distribution patterns across seasons. The APC in crab marinated in soy sauce varied from 4.36 to 5.28 log CFU/g, with the highest mean observed in the fall. Conversely, the CC levels ranged from 1.07 to 2.07 log CFU/g, peaking in the summer. Significant seasonal differences were observed in both APC and CC for the soy sauce samples. Although crab marinated in spicy sauce samples also showed seasonal variation in APC, no significant differences were indicated. In contrast, CC values were highest ($p < 0.05$) in the summer, similar to the soy sauce samples.

3.2.2. Culture-independent microbial community analysis through 16S rRNA sequencing

Fig. 8 examines seasonality variation at the genus level. A total of 1287 genera were identified, with only the top 20 presented for each soy sauce and spicy sauce sample. The microbial community composition exhibited a similar pattern of abundance across all four seasons. As shown in Fig. 8, psychrotolerant bacteria, such as *Psychrobacter* (16.42 %–35.37 %) was predominant across all sample types and seasons, as it can grow within the temperature range of −10 to 42 °C, which covers the temperature conditions for the refrigerated or frozen storage of marinated raw crab (Ayala-del-Río et al., 2010). Consistent with this result, prior research investigating the microbial communities of raw crabs over various seasons (spring and fall) also identified *Psychrobacter* as the predominant genus in all seasons (Anagnostopoulos et al., 2022; S. Kim et al., 2017; Parlapani, 2021). *Psychrobacter* plays a role in organic matter degradation by breaking down lipids and hydrolyzing amino acids and proteins through its unique lipase activity (Broekaert et al., 2013). These cold-active lipases, known for their inherent stability, activity, and flexibility under low-temperature conditions (Maiangwa et al., 2015), contribute to seafood spoilage by degrading lipids and proteins, which leads to the production of volatile compounds.

Table 2
Results of bacterial counts categorized by season.

Sample type	Season	Bacterial counts (log CFU/g)	
		Aerobic plate counts (APC)	Coliform counts (CC)
Crab marinated in soy sauce	Spring	4.36 ± 1.10^{a}	1.07 ± 1.05^{a}
	Summer	4.80 ± 0.69^{ab}	2.07 ± 1.05^{b}
	Fall	5.28 ± 1.39^{b}	1.23 ± 0.94^{a}
	Winter	4.51 ± 0.94^{a}	1.28 ± 1.36^{ab}
Crab marinated in spicy sauce	Spring	5.14 ± 0.66	1.58 ± 1.16^{b}
	Summer	5.43 ± 0.72	2.60 ± 0.88^{a}
	Fall	5.28 ± 0.69	1.82 ± 1.17^{b}
	Winter	5.37 ± 0.55	1.40 ± 1.03^{b}

Different superscript letters indicate significant differences within the same category (where columns intersect with rows), denoting statistical significance at $p < 0.05$. In cases where no significant differences were found, no letters were assigned.

Photobacterium levels spiked in both sauce types during the summer months, with 11.12 % in soy sauce samples and 21.91 % in spicy sauce samples. Most strains of *Photobacterium* exhibit optimal growth at temperatures ranging from 5 to 25 °C (Moi et al., 2017). *Photobacterium*, an SSO that accelerates seafood decay (Dalgaard, 1995), needs careful monitoring and control during warm periods to prevent potential spoilage risks. These bacteria produce H_2S, biogenic amines, and trimethylamine, which contributes to off-flavors, off-odors, slime formation, and overall quality deterioration (G. Wang et al., 2017).

Fig. 8B highlights the LAB among the 20 genera presented in Fig. 8A. Although the optimal growth temperature for most LAB ranges from 30 to 45 °C (Terpou et al., 2019), certain genera—such as *Carnobacterium*, *Enterococcus*, *Vagococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella*, and *Lactococcus*—are capable of growth below 10 °C (Giraffa, 2014; Nicholson et al., 2013; Vinderola et al., 2019). Consequently, while seasonal differences were not highly significant, LAB tended to exhibit higher abundance during warmer seasons. LAB abundance peaked in the fall for crab marinated in soy sauce, whereas crab marinated in spicy sauce showed elevated levels during both the summer and fall seasons. In addition to temperature, other environmental factors—such as pH, salinity, electrical conductivity, turbidity, diet, pathogen infection, water quality, and host development—can affect the bacterial community composition and contribute to the observed variations (S. Kim et al., 2017; Lin et al., 2020; López-Hernández et al., 2015; Shi et al., 2019; Sun et al., 2018). These factors can interact in complex ways, potentially driving the fluctuations observed in this study.

The dominant LABs identified in both types of marinated raw crabs were *Carnobacterium* and *Leuconostoc*. The presence of spoilage-causing or problematic bacteria including *Psychrobacter*, *Photobacterium*, *Vibrio*, and *Staphylococcus* highlighted the need for effective hygiene control. To mitigate the presence of spoilage-causing and pathogenic bacteria, several control strategies are proposed. Lactic acid bacteria utilization may serve as a promising strategy for microbial inhibition through mechanisms such as competitive exclusion and the production of antimicrobial metabolites like lactic acid (Mokoena et al., 2021; Zapaśnik et al., 2022). This approach can be further enhanced by providing favorable conditions for LAB growth or incorporating LAB-rich ingredients during processing, which may contribute to improved microbial stability of the final product.

A significant proportion of bacteria (58.9 %) was shared between both marinades; however, their overall microbial community compositions differed, likely due to differences in the ingredients used. Notably, *Bacillus* and *Weissella* were more prevalent in spicy sauce samples than soy sauce samples, potentially attributable to red pepper powder, a primary ingredient of the spicy sauce. These findings suggest that supplementary

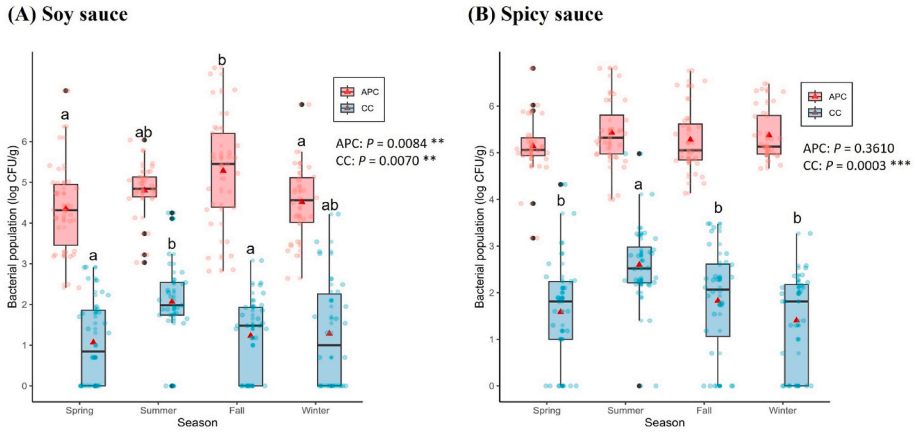


Fig. 7. Aerobic plate counts (APC; pink) and coliform counts (CC; blue) of crab marinated in soy sauce ($n = 120$) and crab marinated in spicy sauce ($n = 120$), categorized by season. The red triangles denote the mean values; each point indicates an individual sample. Different letters above the whiskers indicate significant differences within each count type ($p < 0.05$). In cases where no significant differences were found, no letters were assigned.

ingredients can influence the microbial composition of the final product, emphasizing the importance of ingredient management during processing. Ingredient-specific contamination—particularly from components like red pepper powder—can be addressed through targeted decontamination methods such as pulsed light plasma or electron beam irradiation (Lee et al., 2020; Woldemariam et al., 2021).

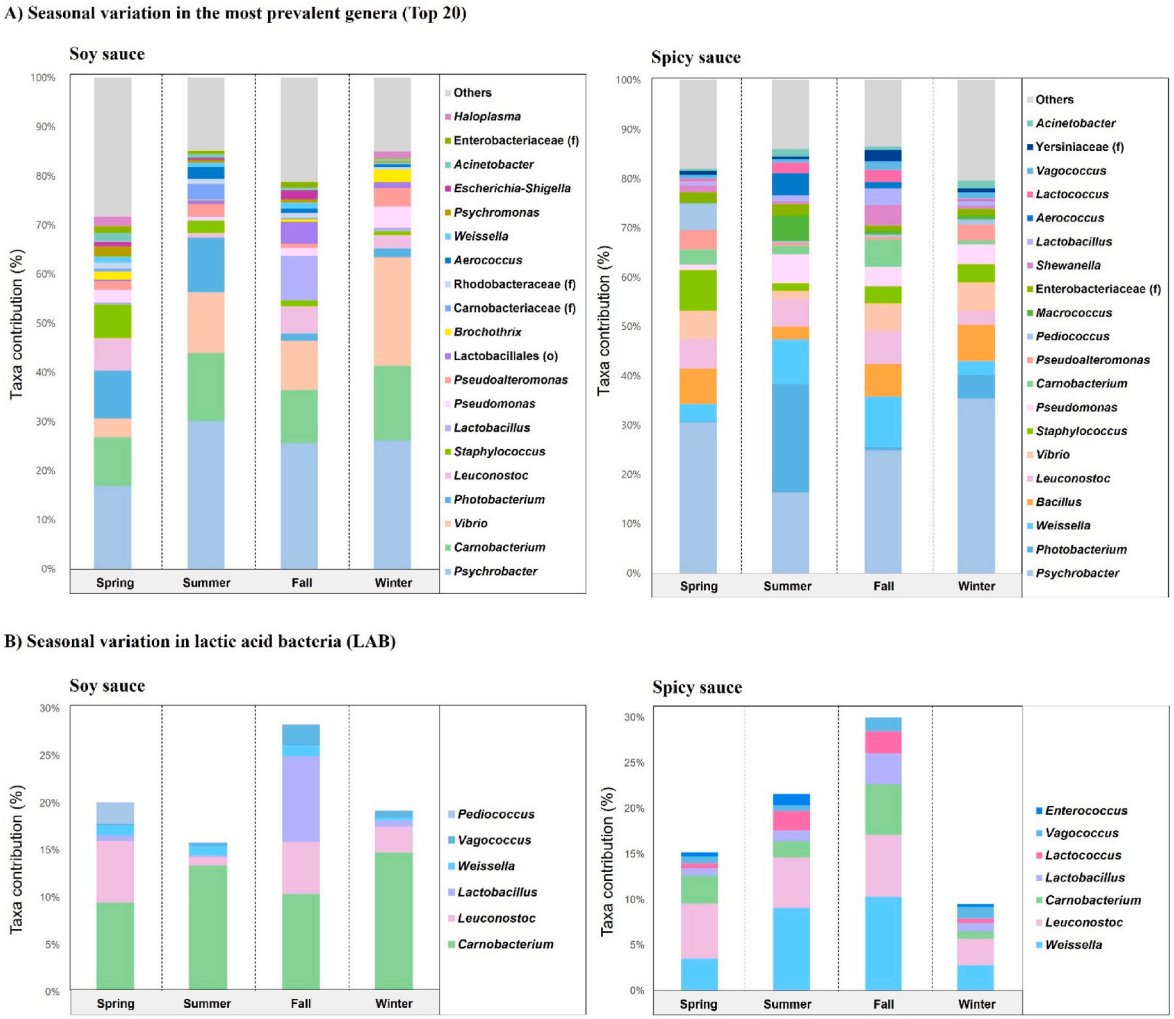


Fig. 8. The *relative* abundance of bacterial genera in crab marinated in soy sauce ($n = 103$) and crab marinated in spicy sauce ($n = 103$), categorized by season. (**A**) The bar charts focus on the 20 most prevalent genera. (**B**) The bar charts focus on lactic acid bacteria (LAB). The size of each segment in the bar chart represents the relative contribution of each genus to the overall microbial community within each sample type across the seasons.

Seasonal variations influenced bacterial composition, notably with *Photobacterium* showing increased abundance during summer months, thereby emphasizing the importance of temperature control during storage. According to Hébraud and Potier (1999), greater temperature fluctuations lead to increased expression of cold shock proteins, which help microorganisms survive under stress. Minimizing temperature fluctuations and maintaining stable low temperatures throughout the supply chain is critical for controlling spoilage. Additionally, psychrotrophic spoilage bacteria can be controlled through strategies such as electron beam irradiation, the use of natural antimicrobial agents (e.g., essential oils or plant extracts), and reducing oxygen availability in packaging. For example, *Pseudomonas* spp. are aerobic microorganisms that require molecular oxygen for growth; thus, vacuum or modified atmosphere packaging can effectively suppress their proliferation (J. Wang et al., 2025).

In conclusion, this study applied HTS technology and culture-dependent methods to investigate the bacterial ecology of commercially distributed crab marinated in soy sauce and crab marinated in spicy sauce. Microbiological analysis revealed that, despite the harsh sauce environments, marinated raw crabs harbor diverse microbial communities. By employing both culture-based methods and sequencing technologies, this study provides a comprehensive microbiological profile of marinated raw crabs and establishes a foundation for future research on microbiome shifts in response to environmental and processing factors.

CRediT authorship contribution statement

Ye Jin Hong: Writing – review & editing, Writing – original draft, Visualization, Methodology, Data curation, Conceptualization. **Yeo Min Kim:** Methodology, Investigation, Data curation. **Young Suk Kim:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Tae Jin Cho:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Min Suk Rhee:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Sun Ae Kim:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.118376.

Data availability

Data will be made available on request.

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