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Biochemical changes induced by dominant bacteria in chill-stored silver carp (*Hypophthalmichthys molitrix*) and GC-IMS identification of volatile organic compounds

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ABSTRACT

To evaluate the spoilage potential of dominant bacteria (*Aeromonas allosaccharophila*, *Pseudomonas psychrophila*, and *Shewanella putrefaciens*) isolated from spoiled silver carp (*Hypophthalmichthys molitrix*) fillets, biochemical changes including protein degradation, trichloroacetic acid (TCA)-soluble peptides, total volatile basic nitrogen (TVB-N), biogenic amines, nucleotide catabolism, and volatile organic compounds were examined in single-species inoculated silver carp flesh for 14 days at 4 °C. *P. psychrophila* exhibited the strongest proteolytic activity, which resulted in the highest concentrations of TCA-soluble peptides and TVB-N. *S. putrefaciens* was responsible for the production of putrescine and cadaverine and led to the fastest degradation of hypoxanthine riboside (HxR). At the end of storage, *P. psychrophila* was the main producer of ketones, especially the C7-C9 ketones, while sulfur compounds were released primarily by *S. putrefaciens*. Moreover, 1-propanol, butanone, 2-hexanone, methyl isobutyl ketone, dimethyl sulfide, and dimethyl disulfide increased gradually with storage time, suggesting their potential as spoilage markers for freshness/spoilage monitoring. *P. psychrophila* possessed the strongest spoilage potential in the fish matrix, followed by *S. putrefaciens*, whereas *A. allosaccharophila* showed a very low spoilage potential. In conclusion, *P. psychrophila* and *S. putrefaciens* were identified as the specific spoilage organisms (SSOs) of silver carp, suggesting that preservation researchers should focus on these two spoilage contributors in future studies. This research contributes to a deeper understanding of silver carp spoilage and to the development of methods and tools to improve fish quality management.

1. Introduction

The popularity of fish products is increasing due to their palatability and nutritional value. In 2016, about 88% of the 171 million tonnes of total fish production were utilized for direct human consumption with the most popular forms being live, fresh or chilled at 45% followed by frozen at 31% (FAO, 2018a). Silver carp (*Hypophthalmichthys molitrix*) is one of the most important farm-raised, freshwater fish species, with a global production of 5,300,736 tonnes in 2016 (FAO, 2018b). However, aquatic products are prone to rapid spoilage and deterioration because of microbial growth, which is mainly due to high moisture concentration, neutral pH, and abundant nutrients (Briones et al., 2010). Bacterial spoilage causes significant economic losses for the fish industry. It is reported that fish losses caused by spoilage are estimated at 10–12 million tonnes per year, accounting for about 10% of the total production from capture fisheries and aquaculture (Tesfay and Teferi, 2017). Therefore, discovering how to restrain the deterioration of aquatic products caused by microorganisms and to extend their shelf-life have become increasingly critical concerns for fish stakeholders.

In order to improve fish quality management, it is necessary to evaluate fish spoilage characteristics. Despite the large number of microorganisms that comprise the initial microbiota of fish, only a few species dominate at the end of shelf-life. When fish become spoiled, the dominant microbiota is comprised of two parts: (i) specific spoilage organisms (SSOs) that become dominant at the onset of spoilage and contribute to the spoilage and (ii) other microorganisms that have

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grown but have not caused unpleasant changes (Gram et al., 2002). According to Gram and Dalgaard (2002), the spoilage potential of a microorganism is the ability of a pure culture to produce the metabolites that are associated with the spoilage of a particular product. Therefore, evaluation of the biochemical changes caused by dominant bacteria in the fish matrix is essential for identifying the SSOs. This knowledge will help focus the future preservation efforts on key target bacteria. During microbiological spoilage of fish, the decomposition of available nutrients and the metabolic activity of spoilage bacteria can lead to the production of volatile organic compounds (VOCs) (Parlapani et al., 2017). Recent researches identified potential spoilage markers in VOCs whose concentrations increased as a function of storage time and microbial growth (Dabadé et al., 2015b; Kuuliala et al., 2018; Parlapani et al., 2019a). The food industry is interested in using VOC markers to indicate freshness and spoilage. Overall, elucidation of SSOs' metabolic potential, and estimation of the growth and population level provide tools for screening of the effective food preservatives *in vitro*, rapid evaluation of fish freshness/spoilage status, and shelf life prediction (Dalgaard, 1995; Koutsoumanis, 2001; Dabadé et al., 2015a; Boziaris and Parlapani, 2017).

Previous studies have demonstrated that SSOs are associated with several undesirable biochemical changes in fish (Macé et al., 2013; Liu et al., 2018). The spoilage microbiota of freshwater fish stored aerobically at 4 °C are generally dominated by *Pseudomonas* spp., *Aeromonas* spp., and *Shewanella* spp. (Huang et al., 2018; Jia et al., 2018a). Spoilage development is a complicated biological event, which needs to be studied at the species and biotype levels (Ercolini et al., 2009). In our previous studies, *A. allosaccharophila*, *P. psychrophila*, and *S. putrefaciens* were identified as the three most common bacterial species of these genera in spoiled silver carp (Jia et al., 2018a). However, the biochemical changes caused by these bacteria in silver carp during chilled storage is still not fully understood. Thus, the aim of the present study was to evaluate the spoilage potential of the three dominant bacteria and to identify the SSOs of silver carp. Protein degradation, biogenic amine accumulation, nucleotide catabolism, and VOCs production were chosen as biochemical indicators to determine the spoilage potential of the isolated bacteria.

2. Materials and methods

2.1. Bacteria strains and growth curve

The three strains used in this study, *P. psychrophila*, *A. allosaccharophila*, and *S. putrefaciens*, were the dominant bacteria that were previously isolated from spoiled silver carp fillets stored aerobically at 4 °C for 6 days (Jia et al., 2018a). Stocks of the strains were stored at −80 °C in tryptic soy broth (TSB, Beijing Aoboxing Bio-Tech, China) with 30% glycerol (Sigma-Aldrich, USA). The strains were pre-cultured individually in TSB at 30 °C for 24 h. Subsequently, each culture (200 μL) was mixed with 20 mL TSB in a 50-mL flask and cultured in a concussion incubator (110 rpm/min) at 30 °C for 9 h. The optical density of the culture at 600 nm was recorded at hourly intervals. Also, the culture was diluted serially (1:10, 0.9% NaCl solution) and 0.1 mL bacterial suspensions were spread onto plate count agars (Beijing Aoboxing Bio-Tech, China) and incubated at 30 °C for 72 h to detect the total viable count (TVC) at different intervals to monitor the growth curves of the bacteria.

2.2. Preparation of sterile fish muscle cubes

Thirty-five silver carp were purchased from an aquatic products market in Beijing, China in May 2018 and transported to the laboratory alive in closed polyethylene bags with water (15 °C) and oxygen. The mean length and weight were 52.70 ± 1.86 cm and 1608 ± 69 g, respectively. Fish were slaughtered ca. 1.5 h after purchase by percussive stunning and were scaled, gutted, beheaded, and washed under running water, after which their outer surface was wiped with 75% ethanol solution. The backbone muscle (with skin) was excised using sterile cutting boards and knives, and all fillets were aired in clean sterile gauze under an ultraviolet radiator for 30 min. Subsequently, fillets were skinned and cut into cubes (about 3 cm × 3 cm × 2 cm; 15–20 g/cube) aseptically. Twelve cubes were obtained from each fish. Cubes were immersed in batches of 30 in 500 mL 0.5% (v/v) formalin solution for 40 s and rinsed with 500 mL sterile water three times. The sterile cubes were stored at 4 °C until all the cubes were sterilized.

2.3. Sample inoculation and storage

To prepare the inoculated samples, strains were pre-cultured in TSB at 30 °C on a concussion incubator until the TVC of bacteria reached 9.0 log CFU/mL. Mixtures of three isolates of the same species were pooled and diluted serially to obtain a microbiological number of 6.0 log CFU/mL. The sterile cubes were divided randomly into four groups: control group (the sterile cubes), *P. psychrophila* group (the sterile cubes inoculated with *P. psychrophila*), *Aeromonas* group (the sterile cubes inoculated with *A. allosaccharophila*), and the *Shewanella* group (the sterile cubes inoculated with *S. putrefaciens*). For each inoculated group, cubes were immersed in batches of 24 in 500 mL inoculation solution for 10 min to achieve an inoculated level of 3–4 log CFU/g. For the control group, fish cubes were immersed in sterile 0.9% NaCl solution. After inoculation, four cubes were packed in each sterile sampling bag (Qingdao Haibo Biotechnology, China) and stored at 4 °C. Three packages in each group were selected randomly and used for analyses on days 0, 3, 6, 8, 10, 12, and 14.

2.4. Enumeration of inoculated bacteria

At each sampling date, three packages of each group were randomly selected and used for microbial enumeration. From each packet, samples were homogenized with 10-fold sterile 0.9% NaCl solution for 30 s in a stomacher (Masticator Basic, IUL Instruments, Spain), and then diluted serially (1:10, 0.9% NaCl solution). Samples (100 μL) of each dilution were spread on a plate count agar and incubated at 30 °C for 72 h. The microbiological number was expressed as log CFU/g.

2.5. Determination of protein degradation

Sarcoplasmic and myofibrillar proteins were extracted according to the method of Liu et al. (2018). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad, USA) was performed using 10% and 12% separating gel with 4% stacking gel for myofibrillar and sarcoplasmic proteins, respectively. Fifteen μg of protein was loaded onto the sample well in the stacking gel, and then electrophoresis was performed at a voltage of 80 V for 20 min, followed by a voltage of 120 V for 40 min. After separation, gels were stabilized in fixative (50% ethanol and 10% acetic acid) for 60 min and subsequently immersed in stainer (10% acetic acid, 50% ethanol, 0.25% Coomassie brilliant blue R-250) for 60 min and destainer (25% ethanol, 8% acetic acid) for 90 min.

2.6. Determination of TCA-soluble peptides

Concentrations of TCA-soluble peptides of silver carp flesh were determined using the method of Yu et al. (2018), with some modifications. Briefly, 3 g of the muscle was homogenized with 27 mL of cold 5% (w/v) trichloroacetic acid (TCA) and stored in crushed ice for 30 min. The obtained homogenate was centrifuged at 10,000 g for 10 min at 4 °C. TCA-soluble peptides were obtained from the supernatant, measured by the Lowry method, and expressed as μmol tyrosine/g.

2.7. Determination of pH, TVB-N and centrifugation loss

Five grams of fish flesh was dispersed in 50 mL distilled water and stirred at 25 °C for 30 min. The mixture was centrifuged at 1610 g for 3 min and the supernatant was collected. A micro-titration method was used to analyze TVB-N with a Kjeldahl Apparatus (KDY-9820, Beijing, China). TVB-N concentrations were expressed as mg N/100 g. The pH of the supernatant was measured using a digital pH meter (Mettler Toledo FE20/EL20, Shanghai, China). Centrifugation loss was also measured in this study. Two grams (W1) of silver carp flesh was weighed and packed with filter paper in a centrifuge tube. Samples were centrifuged at 1760 g for 5 min at 4 °C and weighed again (W2). The centrifugation loss (%) = (W1 – W2)/W1 × 100.

2.8. Determination of biogenic amines

Five grams of minced flesh was homogenized with 10 mL of perchloric acid (0.6 mol/l) and the obtained homogenate was centrifuged at 10,000 g for 5 min at 4 °C, after which the process was repeated once. All the obtained supernatants were combined and adjusted to 25 mL with 0.6 mol/L perchloric acid and stored at −20 °C for further analysis within 2 days. After derivatization with dansyl chloride (Sigma-Aldrich, USA), the ingredient of extract was separated by HPLC (Shimadzu LC-16, Japan) with a COSMOSIL 5C18-PAQ column (4.6 mm × 250 mm), as reported by Huang et al. (2018). Putrescine, cadaverine, and histamine were quantified according to the standard curve.

2.9. Determination of nucleotide degradation

ATP-related compounds (adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine 5′-monophosphate (IMP), hypoxanthine riboside (HxR), and hypoxanthine (Hx)) were extracted according to the method of Huang et al. (2018) and analyzed by HPLC (Shimadzu LC-16, Japan). The separation was performed in a COSMOSIL 5C18-PAQ column (4.6 mm × 250 mm) using phosphate buffer (0.05 mol/L, pH 6.8) that was pumped at 1.0 mL/min. ATP-related compounds were quantified according to the standard curve.

2.10. Determination of VOCs by gas chromatograph - ion mobility spectrometer

Two grams of the minced muscle of each package was placed in a 20 mL headspace vial sealed with crimp caps. The VOCs of fish muscle were assayed with a gas chromatograph - ion mobility spectrometer (GC-IMS) instrument (FlavorSpec®) from Gesellschaft für Analytische Sensorysteme mbH (G.A.S., Dortmund, Germany). The instrument was equipped with a syringe and an autosampler unit for headspace analysis. After 20 min of incubation at 40 °C, 500 μL of sample headspace was injected automatically by using a heated syringe (85 °C) into the heated injector (85 °C) of the GC-IMS equipment. Subsequently, the carrier gas (nitrogen gas) that passed through the injector inserted the sample into the SE-54-CB-1 (5% phenyl-1% vinyl-94% methylpolysiloxane) capillary column (15 m × 0.53 mm, 1 μm film thickness), which was heated at 60 °C for timely separation. After being eluted in the isothermal mode, the analytes were driven into the ionization chamber before detection by the IMS. Molecules were ionized and driven to the drift tube, which was operated at a temperature of 45 °C and a drift gas flow rate of 150 mL/min. Data were processed using the software Laboratory Analytical Viewer (LAV) from G.A.S. Identification of volatiles was achieved by comparing the mass spectrum of samples with those of the NIST 2014 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and IMS mass spectral databases. Retention indexes were calculated by using an n-ketones series (C4-C9) under the same chromatographic conditions as the samples.

2.11. Sensory analysis

Sensory analysis was carried out by six trained and experienced panelists from the lab of aquatic products processing and storage according to Liu et al. (2018) and Macé et al. (2013), with some modifications. In this study, the sensory analysis procedures consisted of a preliminary training and a testing session. During the training session, our sensory panelists figured out the lexicon of sensory descriptors together with specific and univocal definitions. It included characteristics of odor (ammonia, feet/cheese, hydrogen sulfide/putrid odor, acid/vinegar, no off-odor, others), appearance (pink, yellowish, greenish, juice loss, slime), and texture (firm, soft, and extremely soft). The evaluation was performed in a lab equipped with separate booths illuminated with white light. The lighting in the testing area was uniform, free from strong shadows, and controllable. All samples (three packages per group) were placed respectively in transparent and odorless glass bowls covered with lids to keep the odors intact. The samples were assigned individually with three-digit numbers and presented randomly to the panelists. All the panelists evaluated the same samples from four groups. The spoilage grade of silver carp cubes was evaluated by panelists as non-spoiled, lightly spoiled, and strongly spoiled. The samples were considered as spoiled when > 50% of the panelists identified them at the strongly spoiled level.

2.12. Statistical analysis

The GC-IMS was carried out in duplicate. Other measurements were performed in triplicate. Data were presented as mean ± standard deviation. Comparisons of multiple groups and comparisons of different time points were analyzed by one-way analysis of variance (ANOVA) with Duncan's multiple range test using SPSS 22.0 (SPSS Inc., Chicago, IL) software (significance was defined at $P < 0.05$).

3. Results

3.1. Growth curve of bacteria in TSB

The bacterial growth curves were constructed to evaluate the growth of bacteria in TSB (Fig. 1A, Fig. 1B). The amount of *A. allosaccharophila* and *S. putrefaciens* showed a rapid increase after 1 h of incubation in TSB. Meanwhile, *A. allosaccharophila* and *S. putrefaciens* reached a stationary phase after 6 h of incubation with a TVC value of 9.0 log CFU/mL. However, an approximate 4 h of lag phase was observed for *P. psychrophila*, which was about 3 h longer than that of the other two bacteria. *P. psychrophila* showed a sharp increase from 4 h to 5 h of incubation, and it reached a stationary phase after 6 h of incubation in TSB with a TVC value of 9.0 log CFU/mL.

3.2. Enumeration of inoculated strains in silver carp flesh

The TVC of the control was below 2.0 log CFU/g during the entire storage (Fig. 1C), which indicated that the sterile silver carp cubes were prepared successfully. The initial TVC for samples inoculated with *A. allosaccharophila*, *P. psychrophila*, and *S. putrefaciens* were 3.78 ± 0.21, 3.43 ± 0.28, and 3.89 ± 0.23 log CFU/g, respectively. The quantity of *P. psychrophila* and *S. putrefaciens* increased rapidly from day 0 to day 8 and reached their maximum levels of about 9.0 log CFU/g after 10 days of storage. However, compared with *P. psychrophila* and *S. putrefaciens*, *A. allosaccharophila* showed a lower growth rate, and it reached a maximum (8.25 ± 0.26 log CFU/g) at the end of storage. The initial TVC of *P. psychrophila* was lower than that of the other two bacteria in fish flesh. However, *P. psychrophila* grew more rapidly than *A. allosaccharophila* as storage time progressed.

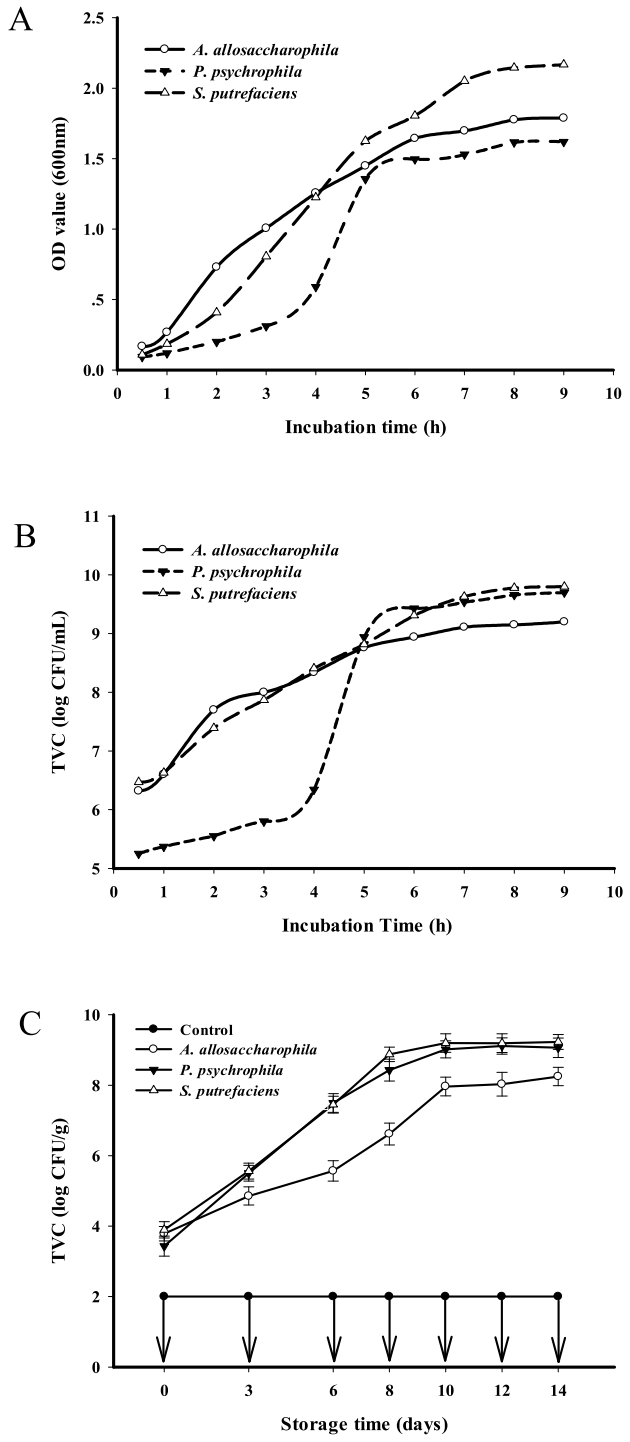

Fig. 1. Bacterial growth curves of *A. allosaccharophila*, *P. psychrophila*, *S. putrefaciens* monitored as (A) optical density at 600 nm and (B) total viable counts (TVC) in tryptic soy broth at 30 °C. (C) Bacterial growth of non-inoculated samples and bacteria inoculated samples during storage at 4 °C. The arrow means that the TVC in non-inoculated samples was below 2.0 log CFU/g during the entire storage.

3.3. Proteolytic activity

Profiles of myofibrillar and sarcoplasmic proteins in the control and inoculated groups showed that there were no obvious changes in myofibrillar and sarcoplasmic proteins among the four groups on day 0 (Fig. 2). Moreover, no significant differences in myofibrillar proteins were observed in the control or samples inoculated with *A. allosaccharophila* or *S. putrefaciens* on day 14 compared with their corresponding initial myofibrillar proteins (Fig. 2A). However, it is noteworthy that significant changes occurred to myofibrillar proteins in the *P. psychrophila* group on day 14. In *P. psychrophila*-inoculated samples, the SDS-PAGE patterns revealed that the band intensity of the myosin heavy chain (MHC, 220 kDa) was reduced dramatically, and two bands with the molecular weight of 25–35 kDa disappeared completely. However, three new bands within 70–130 kDa and a new band with a molecular weight of about 40 kDa appeared. These results indicated that the myofibrillar proteins of silver carp were degraded markedly by *P. psychrophila*. Similar to the results of myofibrillar proteins, little degradation of sarcoplasmic proteins was observed in the control on day 14 (Fig. 2B). In the *A. allosaccharophila* group, there was a decrease in band intensity at 130 kDa. The band with a molecular weight of 40–55 kDa was degraded in the *P. psychrophila* and *S. putrefaciens* groups, and this was coupled with the appearance of two new bands of 25–35 kDa.

3.4. TCA-soluble peptides

The initial concentrations of TCA-soluble peptides in all groups was 5.62–6.12 μmol tyrosine/g sample (Fig. 3A). During the first 8 days of storage, TCA-soluble peptides in all four groups increased slightly, with no significant differences among all groups. However, the concentrations of TCA-soluble peptides in *P. psychrophila*- and *S. putrefaciens*-inoculated samples were significantly higher than those of the control and *A. allosaccharophila*-inoculated samples after 8 days of storage ($P < 0.05$). It is noteworthy that the TCA-soluble peptides in the *P. psychrophila* group showed a sharp increase after 10 days of storage and reached 36.85 μmol tyrosine/g sample on day 14, which was significantly higher than that in *S. putrefaciens* group (15.02 μmol tyrosine/g sample).

3.5. TVB-N, pH, and centrifugation loss

The initial concentrations of TVB-N in the four groups were 8.59–9.47 mg N/100 g (Fig. 3B). The TVB-N concentrations of *P. psychrophila*- and *S. putrefaciens*-inoculated samples increased slowly during the first 8 days, and rose sharply during the later stage of storage. Notably, when compared to the control, significantly higher TVB-N concentrations were observed in the *P. psychrophila* and *S. putrefaciens* group from the 10th and 12th day of storage, respectively ($P < 0.05$). At the end of storage, TVB-N in the *P. psychrophila* and *S. putrefaciens* groups reached respective values of 50.80 and 25.80 mg N/100 g. However, the TVB-N levels of the control and the *A. allosaccharophila* group were 11.23 and 12.97 mg N/100 g at the end of storage, respectively. The initial pH in the four groups was 6.6–6.7 (Fig. 3C). Meanwhile, the pH of the control and samples inoculated with *A. allosaccharophila*, *P. psychrophila*, or *S. putrefaciens* decreased initially to about 6.40 and then increased to 6.60, 6.59, 6.54, and 7.0 at the end of storage, respectively. The pH of samples inoculated with *P. psychrophila* was significantly higher than that of the other three groups from the 10th day of storage ($P < 0.05$). The centrifugation loss of the control and *A. allosaccharophila* group increased gradually, and there was no significant difference between the control and *A. allosaccharophila* groups during the entire storage time (Fig. 3D). However, centrifugation loss of the *P. psychrophila* group and the *S. putrefaciens* group was significantly lower than that of the control after the 8th day of storage ($P < 0.05$), whilst the samples inoculated with *P. psychrophila* showed the lowest centrifugation loss (21.3%) compared with the other groups at the end of storage ($P < 0.05$).

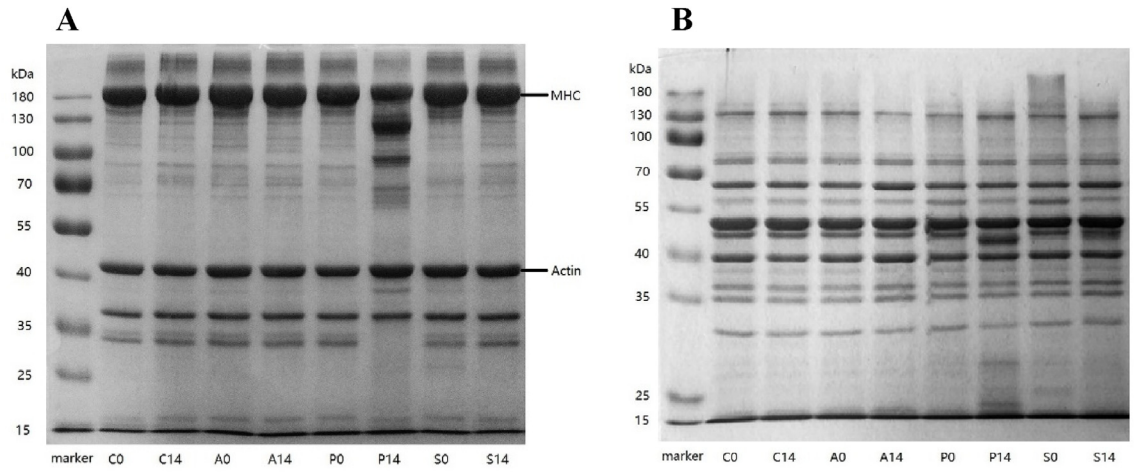

Fig. 2. Profiles of (A) myofibrillar and (B) sarcoplasmic proteins extracted from non-inoculated samples and bacteria inoculated samples. Note: Samples are denoted according to the group (C: non-inoculated; A: *A. allosaccharophila*; P: *P. psychrophila*; S: *S. putrefaciens*) and day of storage (0 or 14). MHC: myosin heavy chain.

3.6. Biogenic amines

Three main spoilage related biogenic amines: putrescine, cadaverine, and histamine were detected and quantified (Table 1). Putrescine was not detected in any group initially, whereas putrescine concentration in the control was detected from the 6th day of storage with a value 0.75 mg/kg, and then it increased gradually to 2.66 mg/kg at the end of storage. *P. psychrophila* and *S. putrefaciens* groups produced significantly higher amounts of putrescine compared with the control during the entire storage ($P < 0.05$), and their putrescine concentrations reached 20.72 and 54.28 mg/kg at end of storage, respectively. However, no significant differences were observed in putrescine

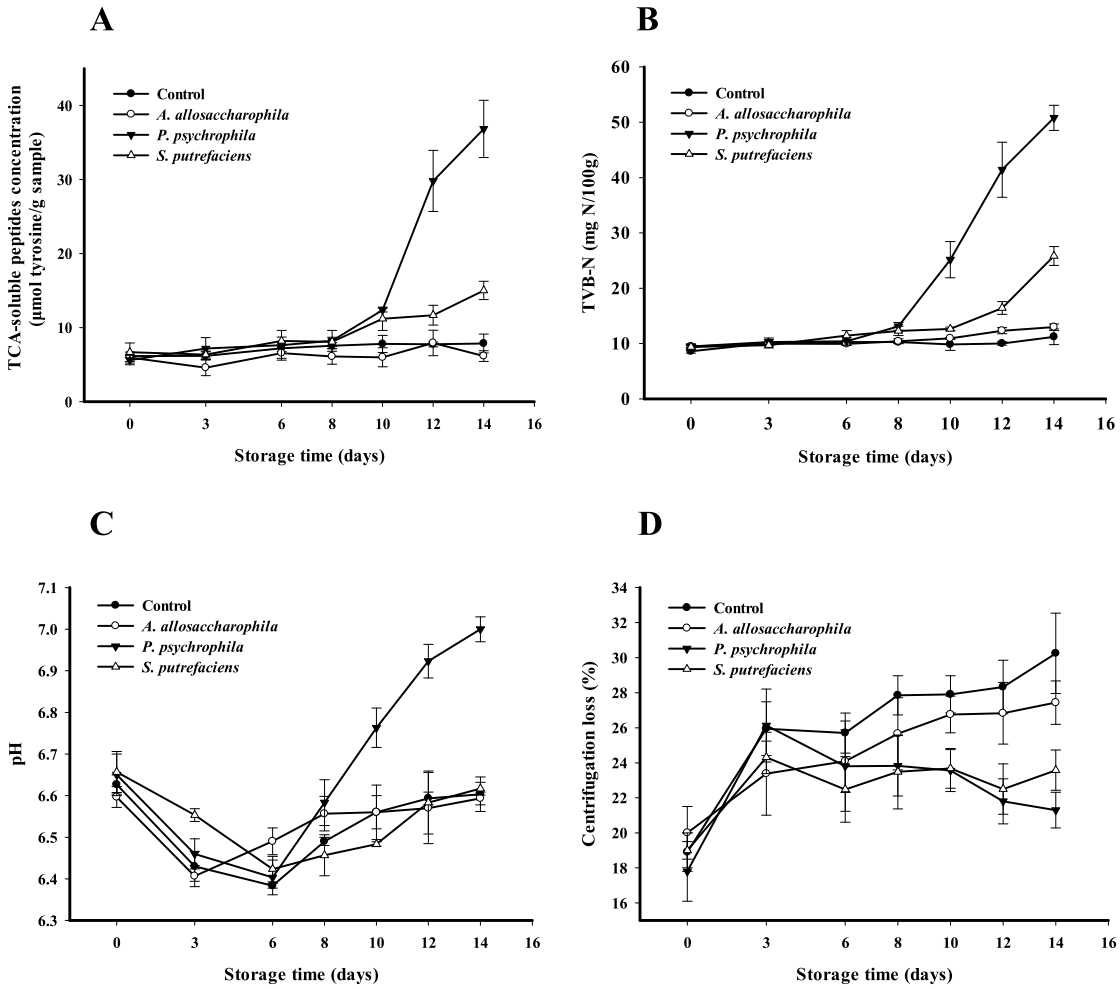

Fig. 3. Changes of (A) TCA-soluble peptides, (B) TVB-N, (C) pH, (D) centrifugation loss in non-inoculated samples and bacteria inoculated samples during storage at 4 °C.

Table 1
Biogenic amines and ATP-related compounds of non-inoculated samples and bacteria inoculated samples during storage at 4 °C.

	Groups	Storage time (day)						
		0	3	6	8	10	12	14
Putrescine (mg/kg)	Control	–	–	0.75 ± 0.24^{Aa}	1.16 ± 0.31^{Aa}	2.16 ± 0.37^{Ab}	2.35 ± 0.79^{Ab}	2.66 ± 0.75^{Ab}
	A. allosaccharophila	–	1.31 ± 0.12^{Aa}	1.56 ± 0.25^{Aa}	3.60 ± 0.54^{Ab}	4.43 ± 1.26^{Abc}	5.42 ± 0.84^{Acd}	5.87 ± 0.55^{Ad}
	P. psychrophila	–	5.68 ± 0.08^{Ba}	6.62 ± 0.80^{Ba}	8.75 ± 0.21^{Bab}	11.83 ± 1.73^{Bbc}	15.14 ± 2.78^{Bc}	20.72 ± 4.15^{Bd}
	S. putrefaciens	–	9.02 ± 0.67^{Ca}	19.37 ± 1.90^{Cb}	26.27 ± 3.39^{Cc}	36.06 ± 7.08^{Cd}	48.50 ± 5.79^{Ce}	54.28 ± 9.83^{Ce}
Cadaverine (mg/kg)	Control	–	–	–	–	–	–	–
	A. allosaccharophila	–	–	–	–	0.24 ± 0.01^{Aa}	0.31 ± 0.01^{Ab}	0.35 ± 0.02^{Ac}
	P. psychrophila	–	–	–	–	–	–	–
	S. putrefaciens	–	–	–	0.52 ± 0.10^{a}	1.87 ± 0.31^{Bb}	3.75 ± 0.45^{Bc}	6.59 ± 1.33^{Bd}
Histamine (mg/kg)	Control	–	–	–	–	–	–	–
	A. allosaccharophila	–	–	–	–	–	–	0.15 ± 0.03^{A}
	P. psychrophila	–	–	–	–	–	0.13 ± 0.07^{Aa}	0.25 ± 0.09^{Aa}
	S. putrefaciens	–	–	–	0.17 ± 0.03^{a}	0.38 ± 0.15^{a}	0.65 ± 0.11^{Bb}	0.93 ± 0.14^{Bc}
IMP (μmol/g)	Control	1.40 ± 0.22^{Ab}	0.25 ± 0.06^{Ca}	0.26 ± 0.04^{a}	–	–	–	–
	A. allosaccharophila	1.66 ± 0.12^{Ab}	0.18 ± 0.05^{BCa}	–	–	–	–	–
	P. psychrophila	1.31 ± 0.13^{Ab}	0.10 ± 0.03^{Aa}	–	–	–	–	–
	S. putrefaciens	1.56 ± 0.12^{Ab}	0.11 ± 0.03^{ABa}	–	–	–	–	–
HxR (μmol/g)	Control	4.04 ± 0.13^{Ba}	4.96 ± 0.86^{Aa}	5.23 ± 0.44^{Ba}	5.18 ± 0.24^{Ba}	4.54 ± 1.11^{Ca}	4.49 ± 0.44^{Da}	5.10 ± 0.41^{Ca}
	A. allosaccharophila	3.75 ± 0.11^{Abc}	5.39 ± 0.60^{Ad}	4.52 ± 0.56^{ABcd}	4.50 ± 0.26^{Bcd}	4.50 ± 0.26^{Ccd}	3.31 ± 0.88^{Cb}	0.96 ± 0.07^{Ba}
	P. psychrophila	4.59 ± 0.23^{Cde}	5.36 ± 0.61^{Ae}	5.00 ± 0.21^{ABde}	4.47 ± 0.56^{Bd}	3.22 ± 0.27^{Bc}	2.27 ± 0.29^{Bb}	0.77 ± 0.08^{Ba}
	S. putrefaciens	4.09 ± 0.36^{Bc}	4.80 ± 0.18^{Ac}	4.25 ± 0.62^{Ac}	1.13 ± 0.43^{Ab}	0.52 ± 0.18^{Aa}	0.20 ± 0.02^{Aa}	0.05 ± 0.04^{Aa}
Hx (μmol/g)	Control	–	0.12 ± 0.03^{ABa}	0.22 ± 0.03^{Ba}	0.26 ± 0.04^{Aa}	0.39 ± 0.09^{Ac}	0.73 ± 0.04^{Ad}	0.76 ± 0.07^{Ad}
	A. allosaccharophila	–	0.11 ± 0.04^{Aa}	0.09 ± 0.04^{Aa}	0.53 ± 0.19^{Ba}	0.56 ± 0.23^{Aa}	2.30 ± 0.55^{Bb}	4.48 ± 0.27^{Cc}
	P. psychrophila	–	0.11 ± 0.03^{Aa}	0.15 ± 0.02^{ABa}	0.71 ± 0.12^{Ba}	2.18 ± 0.99^{Bb}	2.66 ± 0.05^{Bb}	4.27 ± 0.30^{Cc}
	S. putrefaciens	–	0.19 ± 0.06^{Ba}	0.46 ± 0.12^{Ca}	2.48 ± 0.12^{Cb}	2.59 ± 0.38^{Bb}	2.78 ± 0.21^{Bbc}	3.24 ± 0.55^{Bc}

Note: Same uppercase letters in a column indicate no significant differences ($P > 0.05$); same lowercase letters in a raw indicate no significant differences ($P > 0.05$).
— means not detected.

concentration between the control and the samples inoculated with *A. allosaccharophila*. These results indicated that *P. psychrophila* and *S. putrefaciens* were the main producers of putrescine in chill-stored silver carp. Cadaverine was only detected in the *A. allosaccharophila* and *S. putrefaciens* groups after day 10 and day 8, respectively. Cadaverine concentration in the *S. putrefaciens* group reached 6.69 mg/kg at the end of storage, but the concentration of cadaverine in the *A. allosaccharophila* group remained at a low level during the entire storage time and only reached 0.35 mg/kg in the end. Histamine was detected in the *A. allosaccharophila*, *P. psychrophila*, and *S. putrefaciens* groups at the later stage of storage, and it reached a concentration of 0.15, 0.25, and 0.93 mg/kg at the end, respectively.

3.7. Catabolism of nucleotides

To illuminate the effect of bacteria on nucleotide catabolism, changes were evaluated in IMP, HxR, and Hx in sterile samples and samples inoculated with bacteria (Table 1). In our study, the initial IMP concentrations in the control and samples inoculated with *A. allosaccharophila*, *P. psychrophila*, or *S. putrefaciens* were 1.40, 1.66, 1.31, and 1.56 μmol/g, respectively. Despite the fact that IMP concentrations of *P. psychrophila*- and *S. putrefaciens*-inoculated samples were significantly lower than the control and *A. allosaccharophila*-inoculated samples on day 3, IMP was degraded quickly and was not detected after 3 days of storage in inoculated samples. The initial HxR concentrations of the four groups were 3.75–4.59 μmol/g. Along with IMP degradation, HxR concentrations increased initially and then decreased with storage time in all groups. In addition, a sharp decrease in HxR concentration was observed in the *S. putrefaciens* group on day 8, the *P. psychrophila* group on day 10, and the *A. allosaccharophila* group on day 14. At the end of storage, the HxR concentrations of the three inoculated groups remained at very low levels (< 1.0 μmol/g). However, HxR concentration of the control remained at a high level (4.04–5.23 μmol/g) during the entire storage. Moreover, the hydrolysis rate of HxR in samples inoculated with *P. psychrophila* and *S. putrefaciens* were faster than that in *A. allosaccharophila*-inoculated samples. Hx was not detected in the four groups on day 0, but it increased gradually in the four groups after that. Hx concentration in the control remained at a very low level (0.12–0.76 μmol/g) during the entire storage. Along with the degradation of HxR, Hx concentrations showed a sharp increase in the *A. allosaccharophila* group on day 12, in the *P. psychrophila* group on day 10, and in the *S. putrefaciens* group on day 8. At the end of storage, the concentrations of Hx reached 4.48, 4.27, and 3.24 μmol/g for the *A. allosaccharophila*, *P. psychrophila*, and *S. putrefaciens* groups, respectively.

3.8. Volatile organic compounds (VOCs)

In this study, VOCs in the control and inoculated groups were detected on days 0, 8, and 14 by GC-IMS. A total of 25 VOCs was detected, which mainly included alcohols, aldehydes, ketones, esters, and sulfur compounds (Fig. 4, Supplementary Table 1). By comparing the intensity of spot for the profiles of VOCs in the four groups, we determined which compounds increased, decreased, disappeared, or fluctuated during storage (Fig. 4A).

VOCs in the control and in samples inoculated with bacteria were similar on day 0 (Fig. 4). However, types and concentrations of VOCs in all four groups changed at the later stage of storage compared with their corresponding initial VOCs (Fig. 4A and B). New organic compounds appeared in the control samples on day 14 that mainly included alcohols, such as 1-propanol, 1-butanol, 1-hexanol, 1-octen-3-ol, and hexanal. At the end of storage, compared with the control samples, VOCs in the *A. allosaccharophila* group were mainly comprised of alcohols (2-methylpropanol, 1-butanol, 1-octen-3-ol, 1-propanol), ketones (acetone, butanone, 2-hexanone), ethyl acetate, and 3-methylbutanal and accompanied with the decrease of hexanal. Meanwhile, 3-methylbutanal was only detected in the *A. allosaccharophila*-inoculated samples. According to the PCA analysis (Fig. 4C), VOCs in samples inoculated with *A. allosaccharophila* on day 8 and day 14 were similar to those of the control on day 14. Meanwhile, VOCs of *P. psychrophila*- and *S. putrefaciens*-inoculated samples were similar on day 14, though they were distinctly different from those of the control and *A.*

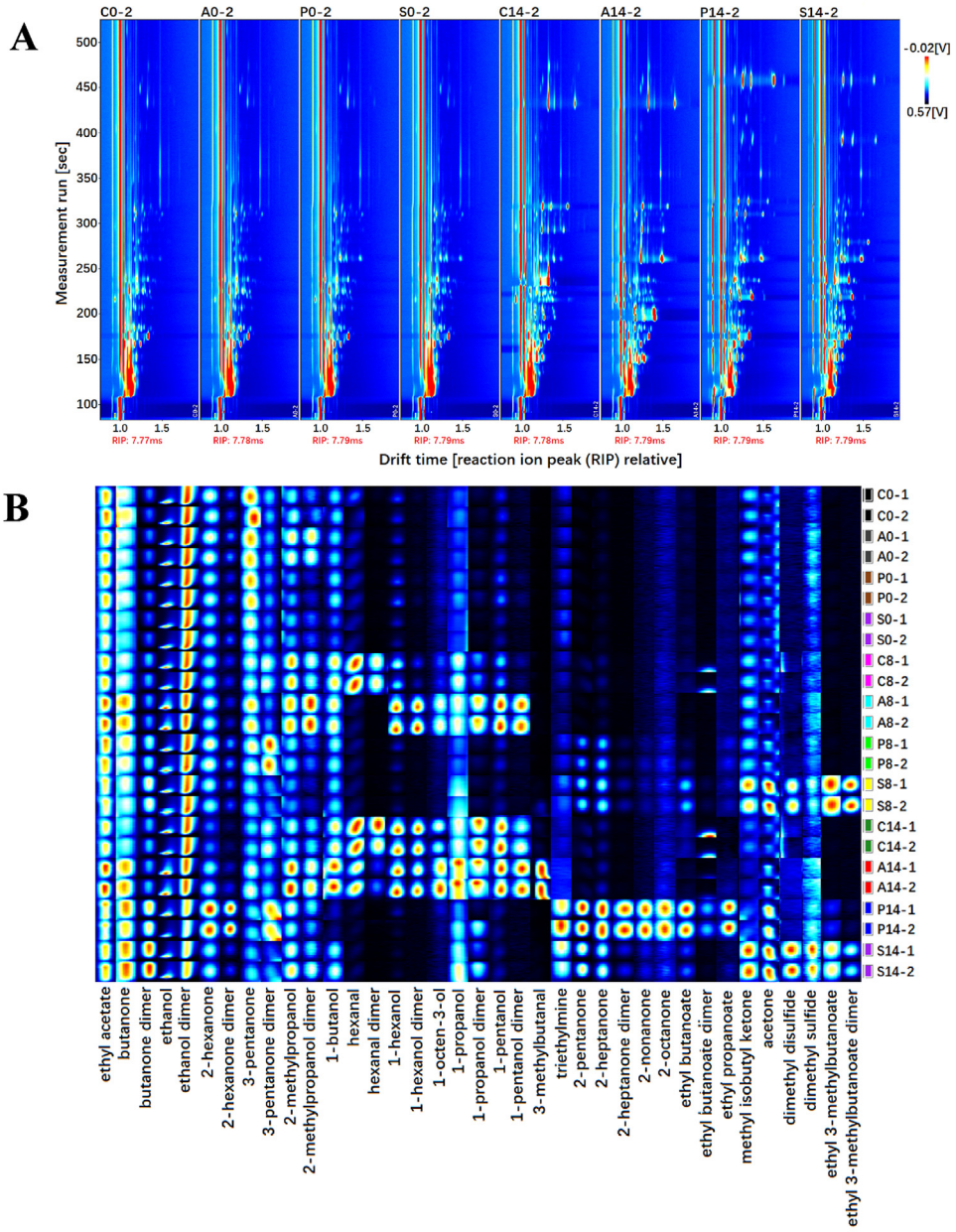

Fig. 4. (A) Topographical plots corresponding to GC-IMS signals detected in non-inoculated control samples and bacteria inoculated samples. (B) Fingerprint comparison of volatiles organic compounds (VOCs) in non-inoculated samples and bacteria inoculated samples determined by GC-IMS. Note: The darker the spot is, the larger is the quantity of volatile compounds. Each row represents all the signal peaks selected in a sample. Each column represents the signal peak of the same volatile compounds in different samples. The GC-IMS was carried out in duplicate. (C) Principal component analysis of VOCs from non-inoculated samples and bacteria inoculated samples. Note: Samples are denoted according to the group (C: non-inoculated; A: *A. allosaccharophila*; P: *P. psychrophila*; S: *S. putrefaciens*) and day of storage (0, 8 or 14).

Table 2
Sensory characteristics of non-inoculated samples and bacteria inoculated samples on day 14.

Groups	Appearance	Texture	Odor
Control	light pink	firm	no off-odor
A. allosaccharophila	light pink	firm	light ammonia
P. psychrophila	juice loss, greenish, slime	extremely soft	strong ammonia
S. putrefaciens	juice loss, yellowish	soft	hydrogen sulfide/ putrid odor

allosaccharophila-inoculated samples. Ketones (acetone, butanone, 3-pentanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone), triethylamine, and ethyl propanoate were abundant VOCs in *P. psychrophila*-inoculated samples compared with the control samples. The results showed that the production of ketones, especially the C7-C9 ketones in silver carp, were associated strongly with *P. psychrophila*. Ethyl propanoate was only detected in *P. psychrophila*-inoculated samples at the end of storage. Triethylamine was also detected in *P. psychrophila*-inoculated samples, which was higher than that in *S. putrefaciens*-inoculated samples. Compared with the control samples, VOCs in *S. putrefaciens*-inoculated samples contained abundant ketones (acetone, butanone, methyl isobutyl ketone), sulfur compounds (dimethyl sulfide and dimethyl disulfide), and ethyl 3-methylbutanoate.

3.9. Sensory analysis

Obvious differences in the sensory index that included appearance, texture, and odor were observed on day 14 among the four groups (Table 2). Samples inoculated with *P. psychrophila* and *S. putrefaciens* were strongly spoiled on day 14, but the control and *A. allosaccharophila*-inoculated samples were still considered as non-spoiled. *P. psychrophila*-inoculated samples exhibited extremely soft texture, which was consistent with the strong proteolytic activity of *P. psychrophila*. Large amounts of surface slime were also observed in *P. psychrophila*-inoculated samples. Moreover, *P. psychrophila*-inoculated samples showed a greenish color. *S. putrefaciens*-inoculated samples displayed strong off-odors that were described as sulfuric. At the end of storage, a yellowish color was observed in *S. putrefaciens*-inoculated samples, though no slime was observed in these samples.

4. Discussion

Fresh fish are among the most perishable food products due to microbial activity. Thus, monitoring and controlling fish quality based on SSOs during fish storage is one of the primary goals for fishery stakeholders. Identification of SSOs and determination of their spoilage domain and spoilage level are necessary to develop preservation techniques. Currently, an increasing number of researchers are working to establish mathematical growth models of SSOs that can predict the progression of spoilage processes in seafood (Koutsoumanis, 2001; Dabadé et al., 2015a), as estimating the residual shelf life at every stage of the food supply chain is essential in improving food quality management. Moreover, rapid and non-destructive detection of fish quality based on SSOs is also a critical issue for microbiology, the fish industry, and the fish quality authorities. Also, detecting the inhibition efficacy of antimicrobials against SSOs *in vitro* is becoming a time-saving approach for screening of effective fish preservatives (Huang et al., 2017). Therefore, in order to identify the SSOs of silver carp and provide the target bacteria for developing fish quality management, we evaluated the spoilage potential of three dominant bacteria isolated from naturally contaminated spoiled silver carp fillets.

In this study, a mix of 3 strains per species was used to avoid a strain effect. The growth trends of three different bacteria were detected in TSB and sterile fish flesh. It is noteworthy that although the lag phase of *P. psychrophila* was 3 h longer than the other two bacteria in TSB, *P. psychrophila* grew more rapidly than *A. allosaccharophila* in sterile fish cubes as storage time progressed. Meanwhile, *P. psychrophila* and *S. putrefaciens* showed a similar growth curve in sterile fish flesh. Several studies have modeled the growth kinetics of SSOs (Lebert et al., 1998; Koutsoumanis, 2001; Dabadé et al., 2015b), however, some of these models were conducted using liquid media under laboratory conditions that do not take into account a potential food matrix effect. Our results indicated that the growth rate of bacteria in fish flesh matrix was different from that in TSB media. Therefore, studies with naturally contaminated actual foods would lead to the accumulation of more reliable information about microbial growth. In our study, the inoculum concentrations of three bacteria were set at 6 log CFU/mL to make sure the initial microbial load in the inoculated sterile fish cubes reached 3–4 log CFU/g which is the initial microbial load in fresh silver carp flesh (Jia et al., 2018a, b). According to Macé et al. (2013), bacterial growth in the fish should be about 6 or 7 log CFU/g to allow enough spoilage metabolite production. In this study, the inoculated silver carp cubes were stored for 14 days to make the spoilage characteristics from the dominant bacteria clearly observable.

In terms of protein degradation, *P. psychrophila* possessed the highest proteolytic activity against the fish protein, especially the myofibrillar protein, compared with *A. allosaccharophila* and *S. putrefaciens*. Previous study indicated that bacterial proteases played an important role in the hydrolysis of meat proteins, which resulted in the release of peptides and amino acids (Pisacane et al., 2015). Venugopal et al. (1983) reported that *Pseudomonas* species produced appreciable amounts of extracellular protease that readily hydrolyzed myofibrillar proteins of Indian mackerel (*Rastrelliger kanagurta*). Wang et al. (2018) determined the proteolytic activity of *Pseudomonas fragi* in chicken meat and reported that myosin was degraded by this bacterium, which was consistent with our study. Softening of fish fillets during chilled storage is believed to be a result of the degradation of muscle proteins, and it is associated with various proteolytic systems of either muscle or microorganisms (Ge et al., 2016). According to the sensory results of our study, obvious softening of fish cubes was observed in *P. psychrophila*-inoculated samples, which was attributed mainly to the hydrolysis of myofibrillar proteins. On the other hand, the sarcoplasmic proteins were degraded by three bacteria in varying levels, and *P. psychrophila* had the highest proteolytic activity against sarcoplasmic proteins among the three bacteria. Bai and Rai (2014) reported that *P. psychrophila* was found to produce protease, additionally, the quorum sensing signaling molecule *N*-butanoyl-L-homoserine lactone (C4-HSL) produced by *P. psychrophila* was capable of inducing protease production. To further determine the effects of bacteria on proteolytic degradation of fish protein, we measured TCA-soluble peptides. TCA-soluble peptides are usually used to monitor protein degradation of postmortem muscle caused by enzymatic hydrolysis or acidic hydrolysis (Benjakul et al., 2003). The present study showed that the initial concentration of TCA-soluble peptides of silver carp flesh was about 6.0 μmol tyrosine/g sample. The initial hydrolysis of muscle proteins was attributed mainly to endogenous cathepsin, followed by the action of microbial peptidases, which further degrades protein into small peptides and free amino acids (Molly et al., 1997). The significant increase of TCA-soluble peptides at the later stage of storage was strongly related to the presence of *P. psychrophila* and *S. putrefaciens*. *P. psychrophila*, however, had a greater ability to produce TCA-soluble peptides than *S. putrefaciens*, which may be due to the strong proteolytic activity of *P. psychrophila*. Such a high proteolytic activity of *P. psychrophila* may have resulted in an increase in muscle-derived nitrogenous degradation products, thereby favoring the proliferation of the bacteria that accelerated the decomposition of proteins (Sriket et al., 2012).

The TVB-N levels of the three inoculated groups showed a similar increasing trend with TCA-soluble peptides. The present work showed that *P. psychrophila* produced significantly higher amounts of TVB-N

than *A. allosaccharophila* and *S. putrefaciens*. Dabadé et al. (2015a) and Liu et al. (2018) reported that *P. psychrophila* and *S. putrefaciens* were active producers of TVB-N. In addition, the increasing trends seen in *S. putrefaciens*-inoculated samples were similar to the results of Wang et al. (2017) and Liu et al. (2018). However, the TVB-N value of samples inoculated with *S. putrefaciens* in our study was different from that in these studies. TVB-N in the *P. psychrophila* and *S. putrefaciens* groups reached the respective maximum values of 50.80 and 25.80 mg N/100 g. Dabadé et al. (2015a) reported that the TVB-N value at the sensory rejection times in *P. psychrophila*-inoculated shrimps (*Penaeus notialis*) ranged between 73 and 85 mg N/100 g. Generally, TVB-N values depend on fish species and are related to the fish non-protein nitrogen concentration, which in turn depends on the type of fish feed, the season and region in which fish have been caught, the size, age, and sex of fish, and on microbial activity (Kilinc and Cakli, 2005; Goulas and Kontominas, 2007). The highest TVB-N concentration in *P. psychrophila*-inoculated samples may be attributed to its potent proteolytic activity, as revealed by our protein proteolysis results in section 3.3.

pH value is a critical indicator to evaluate fish quality. The pH in non-inoculated and bacteria inoculated samples all exhibited first a decreasing trend and then an increasing trend. However, notably, the pH of *P. psychrophila*-inoculated samples was significantly higher than that of the other three groups at the later stage of storage. The initial decreases in pH might be associated with the liberation of inorganic phosphate by the degradation of ATP and the generation of lactic acid by anaerobic glycolysis, while the increases during the later stages of storage might be attributed to the accumulation of basic compounds resulting from autolytic and microbial reactions (Delbarre-Ladrat et al., 2006). On the other hand, *A. allosaccharophila* had no effect on the centrifugation loss during the entire storage time. However, the *P. psychrophila*- and *S. putrefaciens*-inoculated samples showed lower centrifugation loss than that of the non-inoculated samples at the later stage of storage. This was consistent with the results of Wang et al. (2017). Our sensory result showed that *P. psychrophila* released large amounts of surface slime during the entire storage period. The slime, which was composed mainly of polysaccharide, became a protective layer on the surface of the silver carp cubes, which may have made it difficult for water to be centrifuged from the sample (Björkroth and Korkeala, 1997).

Putrescine, cadaverine, and histamine pose significant risks to health when eating fish, and they are useful in determining the quality of fish (Bulushi et al., 2009). According to our results, *P. psychrophila* and *S. putrefaciens* were the main producers of putrescine. Cadaverine was only detected in the *A. allosaccharophila* and *S. putrefaciens* groups at later stage of storage. However, cadaverine concentration in the *S. putrefaciens* group increased more quickly than in the *A. allosaccharophila* group. Visciano et al. (2012) reported that cadaverine was the amine that was most strongly correlated with *S. putrefaciens*, and that putrescine showed the strongest correlation with the genus *Pseudomonas*. Histamine was detected in the *A. allosaccharophila*, *P. psychrophila*, and *S. putrefaciens* groups at the later stage of storage. However, their values were far lower than the Food and Drug Administration (FDA) maximum allowable level of 50 mg/kg (FDA, 1998). The catabolism of nucleotides is also one of the most important biochemical changes in fish after death. The pathway of ATP degradation is documented as a catabolite sequence: ATP→ADP→AMP→IMP→HxR→Hx (Hong et al., 2017). In our study, the initial IMP concentrations in all groups were about 1.5 μmol/g, which was in agreement with the results of Li et al. (2017). The IMP in *P. psychrophila*- and *S. putrefaciens*-inoculated samples showed lower levels than in the non-inoculated samples, whereas IMP in all groups decreased to very low levels during the first 3 days, which was consistent with the results of Liu et al. (2018). Previous studies indicated that IMP showed first an increasing trend and then a decreasing trend during the first 3 days of storage (Huang et al., 2017; Liu et al., 2018). Therefore, to detect the effect of bacteria on changes in IMP levels, researchers must set more detection points during the first 3 days of storage. Along with IMP degradation, HxR in all samples initially increased and then decreased during storage. Our results illustrated that the degradation of HxR was primarily due to the three bacteria rather than autolysis. Notably, *S. putrefaciens* showed the highest hydrolysis rates of HxR to Hx, followed by *P. psychrophila* and *A. allosaccharophila*, which resulted in the accumulation of Hx in the three inoculated groups. Liu et al. (2018) reported that the hydrolysis of HxR to Hx may be attributed to a bacterial hydrolytic enzyme, such as inosine nucleosidase.

Currently, several studies have shown a correlation between spoilage volatile compound release and the development of specific microbial species during storage of seafood such as fish (Wierda et al., 2006; Macé et al., 2013), crab (Parlapani et al., 2019b), and shrimp (Macé et al., 2014). One of the objectives of this study was to investigate the volatile compounds produced by the dominant spoilers to determine spoilage markers. The origin of VOCs during fish spoilage is attributed to either the decomposition of fish constituents or to the metabolic activity of spoilage bacteria (Parlapani et al., 2017). According to our results, the VOCs were changed with time even in sterile fish cubes. New compounds appeared in sterile fish muscle after 14 days of storage and were mainly composed of alcohols (1-propanol, 1-butanol, 1-hexanol, 1-octen-3-ol) and hexanal, which indicated that these VOCs could be produced by nonmicrobial action. Parlapani et al. (2017) reported that various C6-C10 saturated and unsaturated alcohols and aldehydes are examples of VOCs that could be produced by nonmicrobial action.

There were also some volatile compounds produced by microbial activity. Our results demonstrated that the production of ketones (especially the C7-C9 ketones), triethylamine, and ethyl propanoate were strongly associated with *P. psychrophila*. Previous studies also demonstrated that ketones like 2-heptanone, 2-nonanone, and 2-octanone were associated with *Pseudomonas* spp. and were important contributors to the odor of spoiled meat (Ercolini et al., 2010; Parlapani et al., 2017). Ketones in fish may be formed as a result of several fatty acid oxidation reactions, chemical autooxidation, and enzymatic α- or β-oxidation, or be formed by alkane degradation or dehydrogenation of alcohols caused by Gram-negative bacteria (Argyri et al., 2015). *Pseudomonas* spp. was also the major producer of ester in meat (Argyri et al., 2015; Casaburi et al., 2015). Ethyl propanoate was only detected in *P. psychrophila*-inoculated samples at the end of storage. Esters may be produced by esterification of the various alcohols and carboxylic acids in meat depending on microbial esterase activity (Talon et al., 1998). Triethylamine, which is a colorless volatile liquid with a strong ammonia odor, was detected in *P. psychrophila*-inoculated samples at a higher concentration than were found in *S. putrefaciens*-inoculated samples. The generation of C3-C6 ketones, sulfur compounds, and ethyl 3-methylbutanoate was related to *S. putrefaciens*. Sulfur compounds are important components of putrid off-odors and have low odor thresholds (Casaburi et al., 2015). According to our sensory analysis, *S. putrefaciens*-inoculated samples displayed strong off-odors that were described as a hydrogen sulfide/putrid odor. Specifically, sulfur compounds may be due to catabolism of methionine to methanethiol, which is then further oxidized chemically or enzymatically to form the polymeric organic sulfides (Nychas et al., 2007). According to the PCA analysis, VOCs of samples inoculated with *A. allosaccharophila* were similar to those of the non-inoculated samples at the later stage of storage, which was verified by the sensory analysis. These results validated the low spoilage potential of *A. allosaccharophila* in terms of off-odor release. On the other hand, VOCs in *P. psychrophila*- and *S. putrefacient*-inoculated samples were similar to one another, but were distinctly different from those found in the non- and *A. allosaccharophila*-inoculated samples. *A. allosaccharophila* was associated with the production of alcohols, C3-C6 ketones, ethyl acetate, and 3-methylbutanal. 3-methylbutanal was only detected in the *A. allosaccharophila*-inoculated samples at the end of storage. Generally, 3-methylbutanal, which is formed from leucine, is associated with a pungent cheese and

apple-like odor (Casaburi et al., 2015). Jørgensen et al. (2001) reported that 3-methylbutanal contributed to the spoilage and off-flavor of cold-smoked salmon (*Salmo salar*), and this compound has been proposed as a potential fish spoilage indicator (Parlapani et al., 2017). However, the *A. allosaccharophila*-inoculated samples were not considered spoiled at the end of storage, perhaps because there was not enough of 3-methylbutanal to result in off-odor. Several studies have demonstrated that the combination of different compounds rather than a single one can affect the sensory profile of seafood products (Laursen et al., 2006; Jaffrès et al., 2011).

Overall, compounds such as 2-methylpropanol, acetone, butanone, 2-hexanone, 2-pentanone, 2-heptanone, 2-nonanone, 2-octanone, triethylamine, and ethyl propanoate were associated with *P. psychrophila*; 2-methylpropanol, 1-propanol, butanone, acetone, methyl isobutyl ketone, dimethyl sulfide, dimethyl disulfide, and triethylamine were associated with *S. putrefaciens*. These compounds were found to increase at the end of storage. A suitable spoilage marker should be microbial metabolite produced by the spoilage microorganisms. It should initially be absent or at least present at low levels in food, and should increase during storage, showing a strong correlation with microbial growth (Jay, 1986; Parlapani et al., 2017). According to our results, 1-propanol, butanone, 2-hexanone, methyl isobutyl ketone, dimethyl sulfide, and dimethyl disulfide were absent initially and increased gradually with the storage time, showing a good correlation with the microbial growth. Therefore, these compounds had the potential to be spoilage markers for silver carp.

5. Conclusions

This study clarified the significance of isolated dominant bacteria in the biochemical changes associated with the spoilage of chill-stored silver carp. *P. psychrophila* possessed the strongest spoilage potential in the fish matrix among the three bacteria, followed by *S. putrefaciens*, while *A. allosaccharophila* had a very low spoilage potential. Based on our previous study which showed that *P. psychrophila* and *S. putrefaciens* belonged to the initial natural microbiota and eventually became the dominant bacteria of spoiled silver carp (Jia et al., 2018a), we concluded that *P. psychrophila* and *S. putrefaciens* were the SSOs of chill-stored silver carp. Thus, these results suggest that researchers should focus on these two spoilage contributors in future studies regarding the preservation of silver carp. To our knowledge, this is the first study that identified the SSOs in chill-stored silver carp and illustrated their spoilage characteristics, which contributes to a deeper understanding of silver carp spoilage. Finally, further studies based on SSOs (such as shelf life prediction, rapid detection, and antimicrobial screening) need to be conducted to develop methods and tools for the improvement of silver carp quality management.

Declaration of interest statement

All authors declare that there is no conflict of interest related to this article.

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Appendix A. Supplementary data

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