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ORIGINAL ARTICLE

Study on the spoilage potential of *Pseudomonas fluorescens* on salmon stored at different temperatures

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Abstract The bacterial kinetics and quality indexes [sensory quality, total volatile basic nitrogen (TVB-N), thiobarbituric acid value, biogenic amine, and amino acids] were analyzed on salmon inoculated with *Pseudomonas fluorescens* during storage under different temperatures (30, 10, and 4 °C). The bacterial kinetics revealed that *P. fluorescens* showed a steady growth at low temperatures (10 and 4 °C). The TVB-N yield factors of the sample stored at 4 °C indicated that each bacterial cell of *P. fluorescens* displayed greater spoilage activity at low temperatures. A remarkable correlation was found between the production of biogenic amines and bacterial counts. The results also highlighted that *P. fluorescens* cultured at 4 °C had higher demand for amino acids.

Keywords *Salmo salar* · *Pseudomonas fluorescens* · Bacterial kinetic · Spoilage potential · Amino acid degradation

Introduction

Salmon (*Salmo salar*) is one of the most important commercial fish species in the world, which is favored by consumers due to the good flavor and nutritional properties. In particular, salmon is rich in protein and low in cholesterol, but it may provide nutrients for microbial growth. In the process of cold chain logistics, the deterioration of salmon attribute to microbial growth and metabolism, (Gunsen et al. 2011). The dominant microorganisms contributing to the spoilage in raw aquatic products are called the specific spoilage organisms (SSOs) (Gram and Dalgaard 2002). Small molecules such as volatile basic nitrogen, biogenic amines and organic acids are produced by the metabolism of SSOs in raw aquatic products, which could function as indicators of spoilage (Erikson et al. 2011).

The most common spoilage bacteria reported in salmon under aerobic and refrigerated storage conditions are *Pseudomonas* spp., *Carnobacterium* spp., and *Shewanella* spp. (Hatje et al. 2014; Picart et al. 2004; Sabrina et al. 2012). It is reported that *Pseudomonas* spp. was the most important spoilage microorganisms at low storage temperatures (He and Sun 2015), which may lead to the decomposition of nitrogenous substances and the production of ammonia, trimethylamine and hydrogen sulfide in salmon under cold chain logistics (Hu et al. 2013). This may lead to increase of enzymatic activity, denaturation of the muscle proteins and structural damage of membranes, which can result in increased weight loss, reduction of water-holding capacity, textural changes and off-odor. However, the correlation analysis between spoilage characteristics of *Pseudomonas fluorescens* and temperatures in salmon has not been established clearly.

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Therefore, the aim of this study is to investigate the spoilage potential of *P. fluorescens* in salmon fillets stored at different temperatures (30, 10 and 4 °C). To do this, the sterile salmon fillets were inoculated with the *P. fluorescens* strains, and the changes in bacterial counts, sensory quality, total volatile basic nitrogen, thiobarbituric acid value, biogenic amines and total amino acids were determined.

Materials and methods

Bacterial strains

The bacteria strain *P. fluorescens* (NCBI accession no: SBW25) isolated from spoiled salmon previously was stored in sterilized brain heart influsion broth (BHI; Qingdao Hope Bio-Technology Co., Ltd., Qingdao, PR China) and glycerine (1:3) at − 80 °C. Before use, this bacteria strain was precultured in BHI at 30 °C for 18 h and then cultured in Tryptose soya broth (TSB; Qingdao Hope Bio-Technology Co., Ltd., Qingdao, PR China) at 27 °C until the counts reached 10^8 CFU/ml (Churchill et al. 2016).

Sample preparation and inoculation

Salmon sample purchased from a local aquatic market in Shanghai was transported in a foam box contained ice to laboratory within 30 min. Then the salmon sample was gutted and cut into fish fillets (60–75 g). The fillets were sterilized by soaked in 75% ethanol solution for 60 s and washed in sterile water 2 times (Qian et al. 2014). The precultured bacteria strain solution was applied for the inoculation process. Each sterile fillet was immersed in a bacterial suspension (10^5 CFU/g) for 30 s. Non-inoculated fillets washed by sterile water were used as the control. All samples were drained for 10 s, packed in clean tray in a sterile environment and stored at 30, 10, and 4 °C, respectively. The related indexes were measured periodically during storage.

Sensory evaluation

The salmon samples were displayed on the clean sterile tray. The sensory characteristics were according to the method described by Sallam (2007). Ten trained panelists from the laboratory staff were requested to assess the general likeness of salmon fillets through appearance, odour, colour, dehydration and firmness independently. The scores were given in the decreasing order scale: 10–9 for excellent, 8–7 for good, 6–5 for acceptable, 4–3 for poor and 2–1 for very poor. The panelists should be familiar with the rating scales beforehand.

Microbiological analysis

Quantitative microbiological analysis were determined based on the method of Song et al. (2011). A 25 g of salmon fillets was aseptically weighed and homogenized with 225 ml sterilized saline water (0.85%, w/v) for 1–2 min. Then the homogenized sample was serially diluted (1:10) in sterile saline water for bacteriological analysis. The samples of 1 ml suitable dilution were inoculated in the plate count agar (PCA, Qingdao Hope Biol-Technology Co., Ltd., Qingdao, PR China) and Pseudomonas CFC selective agar (Qingdao Hope Biol-Technology Co., Ltd., Qingdao, PR China) separately. The plates of CFC selective agar were incubated at 30 °C for 2 days for the enumeration of *Pseudomonas* spp., and the plates of PCA were inoculated at 4 °C for 10 days for psychrotrops.

Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was analyzed according to the method of Uriarte-Montoya et al. (2010). Five grams of minced meat from each sample was accurately weighed and measured by an Automatic Kjeldahl Apparatus (KjeltecTM8400; FOSS Quality Assurance Co., Ltd., Denmark). TVB-N values were expressed as mg N/100 g.

Thiobarbituric acid value (TBA)

The determination of TBA value was performed according to Rode and Hovda (2016). Salmon samples (5 g) were homogenized with 25 ml of 20% (w/v) trichloroacetic acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, PR China) and incubated at room temperature for 1 h. Then the samples were centrifuged at 8000*g* and 4 °C for 10 min. The supernatant was filtered and diluted with deionized water to the volume of 50 ml. Five milliliters of the solution was mixed with 5 ml 0.02 M thiobarbituric acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, PR China). The tubes were incubated in a water bath at 100 °C for 20 min. After cooling to room temperature, the absorbance was determined at 532 nm, and the TBA values were calculated according to the formula.

$$\text{TBA value} = 7.8 \times \text{OD}_{532}$$

The results were presented as mg malonaldehyde/kg of samples.

Biogenic amine (BA)

The extraction and derivatization of biogenic amines for samples were performed according to the measures of Shukla et al. (2010) with some modifications. The mixture was filtered through a 0.45 μm membrane and stored at − 25 °C before analysis. The quantification of BA was measured by HPLC system (LC-2010C series, Shimadzu Co., Ltd., Japan). BA was separated by a C18 column (C18-WP, 4.6 mm × 150 mm, 5 μm, Shimadzu Co., Ltd., Japan). The solvents used as the mobile phase were 0.1 M ammonium acetate (A) and 0.1 M acetonitrile (B). The gradient program was described as following: 0–7 min, 45%A–50%A, 55%B–50%B; 7–25 min, 50%A–10%A, 50%B–90%B; 25–35 min, 10%A–45%A, 90%B–55%B; 35–45 min, 45%A–10%A, 55%B–90%B. The flow rate was 1 ml/min and the column apparatus were at 40 °C. A sample volume of 10 μl was injected and detected at 254 nm.

Total amino acid (TAA)

The TAAs of salmon samples were determined using an automatic amino acid analyzer (Hitachi Global Co., Ltd., Japan) (Deng et al. 2015). Salmon meat samples (100 mg) were homogenized with 6 ml hydrochloric acid (6 M) and hydrolyzed at 120 °C for 24 h. The hydrolysate was diluted to 50 ml by citrate buffer (pH 2.2) and 2 ml of the sample was filtered through 0.45 μm membrane before injecting to the analyzer.

Spoilage potential evaluation

The microbial colonies and the contents of, TVB-N were recorded. The yield factors were calculated as the following formula (Xu et al. 2011):

$$Y_{TVB-N/CFU} = \frac{(TVB-N)_i - (TVB-N)_0}{(CFU)_i - (CFU)_0}$$

where $(TVB\text{-}N)_0$ and $(TVB\text{-}N)_i$ represent the initial contents and the contents of TVB-N at sampling; $(CFU)_0$ and $(CFU)_i$ represent the initial and bacterial counts of spoilage products at sampling.

Statistical analysis

All measurements were carried out in triplicate. Significance of differences defined at $P \leq 0.05$ was used for all means data. Analysis of variance (ANOVA) and mean comparison between the data was evaluated statistically by using SPSS Version 15.0 for Windows (Inc., Chicago, IL, USA). Origin Pro V8.6 (Origin Lab, USA) was applied to render the data graphs.

Results

Changes in sensory quality

Changes in sensory quality of salmon at three different temperatures are shown in Table 1. The initial scores in sensory were 10. The sensory scores of samples at 30 °C decreased rapidly than samples stored at 10 and 4 °C, including the control group. The sensory rejection time in the inoculated group was described as flows: 30 °C for 18 h, 10 °C for 72 h, 4 °C for 144 h. The sensory scores of samples inoculated with *P. fluorescens* were lower than the control.

Bacterial kinetic

The evolution of *P. fluorescens* and psychrotrops at 30, 10 and 4 °C during the salmon storage is shown in the Fig. 1. During storage, the inoculated fillets showed higher bacterial counts than the control. The initial concentrations of inoculated samples and control samples were 4.9 and 3.6 log CFU/g. At 30 °C, *P. fluorescens* counts increased slowly in the first 6 h. Afterward, the concentration rapidly reached to 8.7 log CFU/g and there were no considerable differences between the inoculated and control until the end of storage. At 10 °C, inoculated group increased to 8.6 log CFU/g while control reached 6.8 log CFU/g. At 4 °C, *P. fluorescens* showed a lag phase of 20 h, then it increased rapidly from 20 to 72 h. The growth behavior of psychrotrops was similar with *Pseudomonas* spp.

Changes of total volatile basic nitrogen (TVB-N)

Variable production of TVB-N in salmon at three temperatures was observed in Fig. 2. The TVB-N values of inoculated samples at 30, 10, and 4 °C showed a significant increase after 3, 60 and 96 h. After 24 h, inoculated samples stored at 30 °C exceeded the limit of 30 mg N/100 g. A maximum TVB-N production was observed in inoculated samples at 10 °C for 120 h. However, the control samples stored at 4 °C remained low level of TVB-N (22.39 mg N/100 g) at the end of storage.

Changes of thiobarbituric acid value (TBA)

The changes in TBA under three temperatures (30, 10 and 4 °C) are listed in Table 2. The initial value of control and inoculated salmon fillets were very low (0.046 and 0.093 mg/kg, respectively). During the storage, TBA increased with the time at different temperatures. The TBA of fillets that inoculated with *P. fluorescens* increased rapidly than the control. At the end of storage, the TBA

Table 1 Sensory score of *P. fluorescens* inoculated on salmon fillets under different temperatures

Storage time (hours)	Batches					
	CK30	P30	CK10	P10	CK4	P4
0	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}
6	8.11 ± 0.55^{b}	7.89 ± 0.27^{b}	–	–	–	–
12	6.56 ± 0.93^{c}	6.05 ± 0.71^{c}	8.97 ± 0.90^{b}	8.10 ± 0.47^{b}	–	–
18	5.20 ± 0.62^{d}	4.02 ± 0.82^{d}	–	–	–	–
24	2.06 ± 0.19^{e}	1.17 ± 0.31^{e}	7.52 ± 0.29^{b}	7.55 ± 0.49^{bc}	8.95 ± 0.61^{b}	8.64 ± 0.75^{b}
36	–	–	6.67 ± 0.27^{c}	6.42 ± 0.58^{c}	–	–
48	–	–	5.89 ± 0.32^{d}	5.61 ± 0.92^{cd}	7.49 ± 0.54^{c}	7.12 ± 0.42^{c}
60	–	–	5.49 ± 0.82^{de}	5.03 ± 0.39^{d}	–	–
72	–	–	4.09 ± 0.94^{e}	3.01 ± 0.35^{e}	6.42 ± 0.49^{d}	6.03 ± 0.29^{d}
84	–	–	3.51 ± 0.21^{e}	3.08 ± 0.52^{e}	–	–
96	–	–	–	–	5.51 ± 0.52^{e}	5.19 ± 0.68^{e}
108	–	–	–	–	–	–
120	–	–	–	–	5.30 ± 0.24^{e}	5.12 ± 0.44^{e}
132	–	–	–	–	–	–
144	–	–	–	–	5.07 ± 0.39^{e}	4.61 ± 0.37^{ef}
168	–	–	–	–	4.55 ± 0.24^{ef}	3.41 ± 0.33^{f}
192	–	–	–	–	3.92 ± 0.39^{f}	3.36 ± 0.50^{f}

CK30, CK10, and CK4 represent the non-inoculated samples stored at 30, 10 and 4 °C, respectively, while P30, P10, and P4 represent the inoculated samples stored at 30, 10 and 4 °C, respectively

Different letters in the same column indicated the significant differences between each batches ($P < 0.05$)

"—": Not determined

values of the control samples reached 1.221, 0.592 and 0.235 mg/kg, respectively, and the inoculated samples were 1.358, 0.752 and 0.302 mg/kg, respectively.

Variation of putrescine and cadaverine

A rapid increase of cadaverine and putrescine was observed in the most samples during the storage at higher temperatures (Fig. 3). The initial values of putrescine and cadaverine were 11.31 and 10.51 mg/kg, respectively. The differences of cadaverine between the inoculated samples and the control became significant after 60 h at 10 °C and 96 h at 4 °C, respectively. At the end of storage, the values of putrescine from samples inoculated by *P. fluorescens* stored at 30 °C for 24 h, 10 °C for 108 h and 4 °C for 192 h increased to 235.77, 148.21, and 58.34 mg/kg respectively, while the control were only 189.25, 129.12, and 48.38 mg/kg, respectively. The values of cadaverine of inoculated samples also reached 137.98, 91.25 and 42.15 mg/kg after storage, while the control samples were only 120.89, 51.28, and 28.13 mg/kg respectively.

Changes of total amino acids (TAAs)

The contents of TAA in inoculated samples and non-inoculated samples that stored at 30 °C for 18 h, 10 °C for 60 h and 4 °C for 144 h are depicted in Table 2. The initial total contents of TAA were 212.03 mg/g. A significant decrease of TAA in the inoculated samples and the control was observed. Among them, the content of lysine decreased significantly from 19.60 to 13.19, 12.88 and 12.32 mg/g respectively in inoculated samples. The variation trend of arginine was similar with that of lysine, which decreased from 15.81 to 11.54, 13.18, and 9.43 mg/g.

Discussion

The spoilage potential of *P. fluorescens* on salmon at different temperatures was evaluated. In general, a significant decline was observed in the sensory properties during storage, especially for samples stored at 30 °C. The decreasing rate of the inoculated samples was higher at three temperatures in comparison with the control. At the initial period, no significant differences in sensory quality between the inoculated samples and the control were observed. After 144 h, the samples stored at 4 °C reached

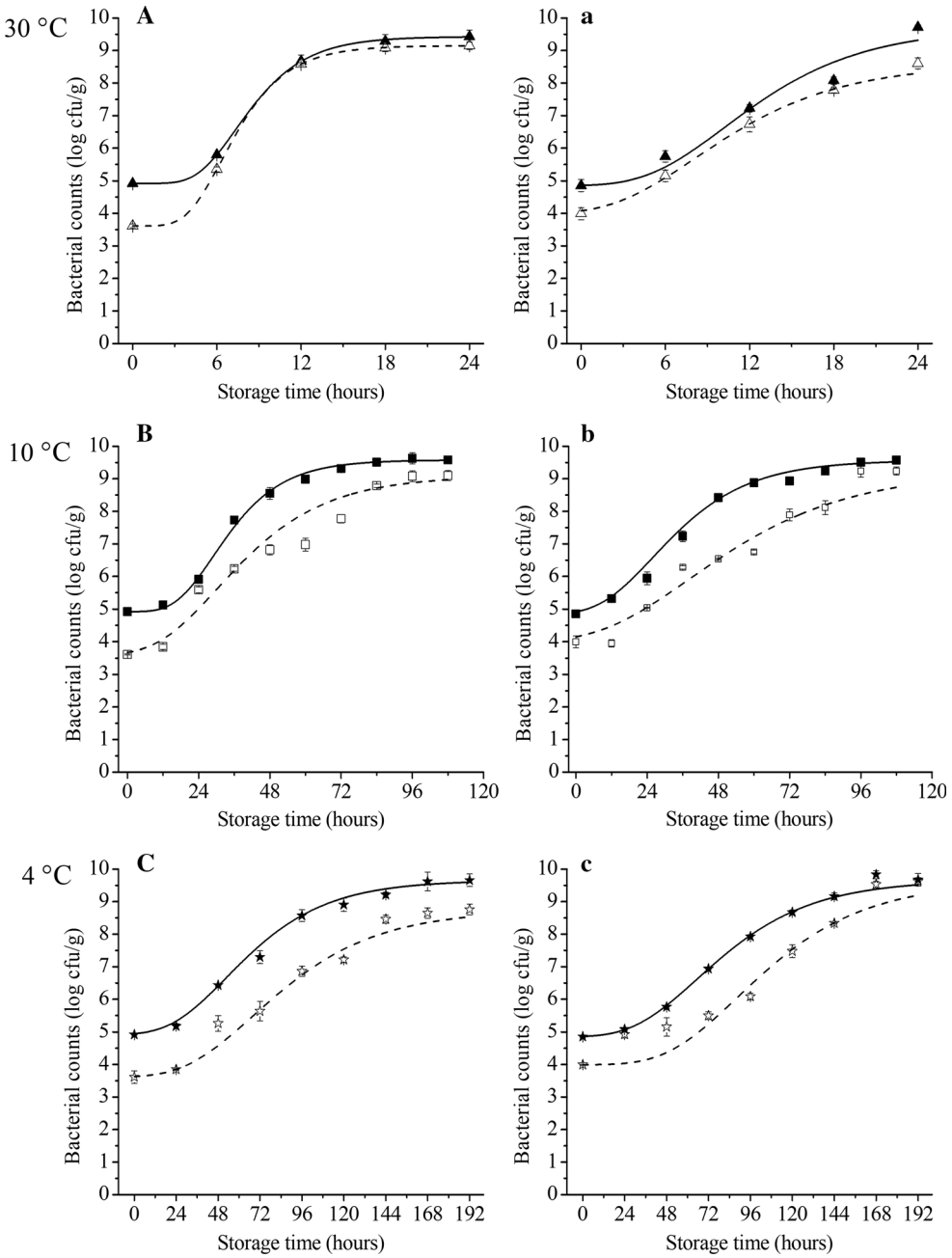

Fig. 1 The fitting growth curve of *P. fluorescens* (A, B, C) and psychrotrops (a, b, c) of salmon stored at 30 °C (open triangle: control, filled triangle: inoculated sample), 10 °C (open square: control, filled square: inoculated sample) and 4 °C (open star: control, filled star: inoculated sample)

the sensory rejection point because of the production of exudates, off-odor, less firmness and more viscosity than the control. An intense dark orange colour of fillets was observed at 4 °C due to the oxidation of astaxanthin and carotenoids, similar to 30 °C. The efficient metabolic capacity of *P. fluorescens* was probably responsible for the modification because they produced TVB-N and BAs. It has been reported that the small molecular proteins in fillets were consumed by *P. fluorescens* (Cardinal et al. 2001). This result confirmed the previous study that *P. fluorescens* showed high protein hydrolytic ability at low temperatures.

The high temperature (30 °C) was considered to be the optimal temperature for the strain of *P. fluorescens* to grow (according to the pre-experiment, data not shown), while the low temperatures (10 and 4 °C) were regarded as the condition of cold storage. In general, the data suggested that *P. fluorescens* at 30 °C showed a shorter lag phase (3 h) and higher growth rate (0.81 CFU/h). Compared to the strains cultured at low temperatures (10 and 4 °C), variations of lag time (3–20.3 h) and maximum growth rate (0.81–0.05 CFU/h) were observed. The sterilizing treatment by 75% ethanol for 60 s could reduce the initial bacterial counts, so that the background bacteria (control

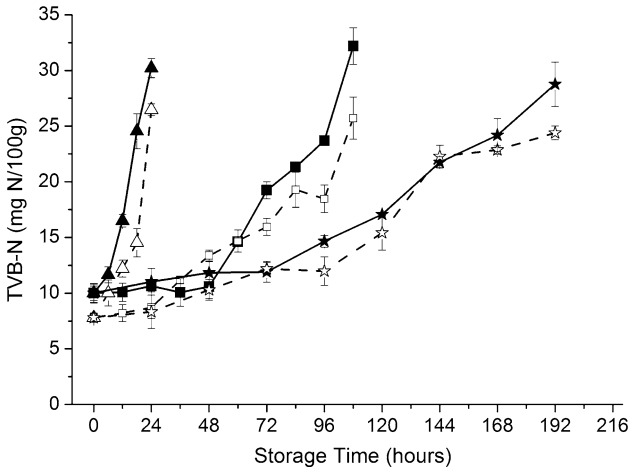

Fig. 2 Changes of total volatile basic nitrogen of salmon stored at 30 °C (open triangle: control, filled triangle: inoculated sample), 10 °C (open square: control, filled square: inoculated sample) and 4 °C (open star: control, filled star: inoculated sample)

group bacteria) only accounted for 4% of the inoculated *P. fluorescens* initially. During the whole culturing period, the counts of background bacteria were lower than that of inoculated *P. fluorescens* and only accounted for 12.5% at 4 °C at the end of storage.

The psychrotrophic growth dynamics were displayed in Fig. 1B. The proportion of psychrotrophic bacteria in salmon may be responsible for the spoilage of salmon during cold storage. In this work, the behavior of psychrotrops was similar to *P. fluorescens*, which indicated that *P. fluorescens* probably was the dominate group of spoilage bacteria at low temperature. This was in agreement with the results of Li et al. (2015). In addition, when bacteria, including *P. fluorescens*, were exposed to stress of low temperatures, a cold-adapted mechanism may occur in order to maintain microbiological activity (Serio et al. 2011).

TVB-N, a nitrogen volatile substance produced by protein decomposition, is widely used as an important indicator of the freshness of aquatic products. It can be found in Fig. 2 that TVB-N value gradually increased significantly with the time. The initial TVB-N value of fresh fillets (7.8 mg N/100 g) was in agreement with low initial bacterial counts. The results showed that TVB-N in the inoculated group was higher than the control group, especially at 10 °C, which indicated that *P. fluorescens* remained high TVB-N metabolic activity at low temperatures. At the end of storage, only inoculated groups achieved a high level of values at three temperatures,

Table 2 Thiobarbituric acid value of *P. fluorescens* inoculated on salmon fillets under different temperatures

Storage time (hours)	Batches					
	CK30	P30	CK10	P10	CK4	P4
0	0.046 ± 0.000^{d}	0.093 ± 0.000^{e}	0.046 ± 0.000^{g}	0.093 ± 0.000^{f}	0.046 ± 0.000^{h}	0.093 ± 0.000^{g}
6	0.218 ± 0.025^{c}	0.327 ± 0.017^{d}	–	–	–	–
12	0.531 ± 0.093^{b}	0.823 ± 0.141^{c}	0.062 ± 0.090^{f}	0.127 ± 0.087^{f}	–	–
18	1.039 ± 0.112^{a}	1.117 ± 0.102^{b}	–	–	–	–
24	1.221 ± 0.129^{a}	1.358 ± 0.091^{a}	0.081 ± 0.039^{b}	0.156 ± 0.043^{ef}	0.048 ± 0.002^{h}	0.080 ± 0.005^{f}
36	–	–	0.191 ± 0.003^{d}	0.225 ± 0.058^{e}	–	–
48	–	–	0.159 ± 0.022^{e}	0.314 ± 0.102^{d}	0.066 ± 0.005^{g}	0.095 ± 0.003^{e}
60	–	–	0.248 ± 0.042^{d}	0.353 ± 0.059^{d}	–	–
72	–	–	0.319 ± 0.074^{c}	0.482 ± 0.085^{c}	0.078 ± 0.004^{f}	0.117 ± 0.012^{d}
84	–	–	0.531 ± 0.031^{b}	0.648 ± 0.022^{b}	–	–
96	–	–	0.592 ± 0.091^{a}	0.752 ± 0.202^{a}	0.094 ± 0.010^{e}	0.158 ± 0.028^{bc}
108	–	–	–	–	–	–
120	–	–	–	–	0.120 ± 0.034^{d}	0.195 ± 0.064^{b}
132	–	–	–	–	–	–
144	–	–	–	–	0.151 ± 0.009^{c}	0.215 ± 0.097^{ab}
168	–	–	–	–	0.181 ± 0.041^{b}	0.267 ± 0.024^{a}
192	–	–	–	–	0.235 ± 0.027^{a}	0.302 ± 0.054^{a}

CK30, CK10, and CK4 represent the non-inoculated samples stored at 30, 10 and 4 °C, respectively, while P30, P10, and P4 represent the inoculated samples stored at 30, 10 and 4 °C, respectively

Different letters in the same column indicated the significant differences between each batches ($P < 0.05$)

"—": Not determined

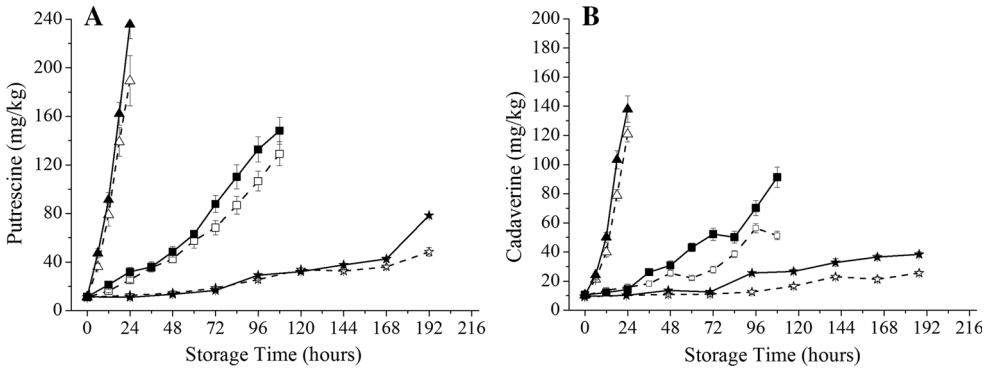

Fig. 3 Variation of the putrescine (**a**) and cadaverine **b** of salmon stored at 30 °C (open triangle: control, filled triangle: inoculated sample), 10 °C (open square: control, filled square: inoculated sample) and 4 °C (open star: control, filled star: inoculated sample)

which can be attributed to the higher bacterial counts. Therefore, *P. fluorescens* might generally be regarded as an effective spoilage bacterium in this study. The changes in TVB-N values in the present work in salmon are similar to the work of Barraza et al. (2015).

A good correlation was observed between TVB-N value and bacterial counts during storage at different temperatures. In order to describe the correlation quantitatively, the yield factor was used to display the production of TVB-N per bacterial cell in inoculated samples. The spoilage potential was evaluated as log $Y_{TVB-N/CFU}$ value (Fig. S1). After the same storage period, higher log $Y_{TVB-N/CFU}$ were observed in samples stored at lower temperatures. After 24 h, the log $Y_{TVB-N/CFU}$ value of samples stored at 4 °C was twice higher than the samples at 30 °C. Therefore, each cell of *P. fluorescens* displayed higher ability to produce TVB-N at low temperatures (10 and 4 °C) than at high temperature (30 °C).

As a widely used indicator of lipid oxidation, the value of TBA expressed as production of malondialdehyde (MDA) (Guillén-Sans and Guzmán-Chozas 1998). The initial TBA value of the inoculated samples was 0.093 mg/kg, which increased to 1.21, 0.752, and 0.302 mg/kg at the end of storage for 30, 10, and 4 °C. In this study, all samples showed an increase in TBA with storage time, including the control. Compared to the control group, *P. fluorescens* showed a slightly higher level of TBA value. Similar results were reported by Amanatidou et al. (2000). We assume that *P. fluorescens* might play a less important role in lipid oxidation.

Biogenic amines (BAs), a low molecular weight organic compounds with biological activity, are produced by the decarboxylation of amino acids (Kim et al. 2009). The most widely studied biogenic amines are histamine, cadaverine, and putrescine among aquatic products and a high level of microbial growth may easily lead to the immoderation of biological amine content (Silla Santos 1996; Sims et al. 1992). According to this study, cadaverine and putrescine were considered as the dominant BAs in salmon samples. *P. fluorescens* was active producer of BAs that were mentioned above. This work was in agreement with the literature (Rezaei et al. 2007), which reported that *Pseudomonas* spp. had a good linear correlation with the formation of cadaverine and putrescine. The accumulation of biogenic amines also can lead to food poisoning. Therefore, cadaverine and putrescine are recommended as the indicators of measuring safety and quality of white-muscle aquatic products such as salmon. (Prester 2011). BAs and other metabolites products could lead to changes in odor. In this study, putrescine and cadaverine showed a great correspondence with the sensory and microbial activity, in accordance with results obtained by Krizek et al. (2004). With the storage time, the production of cadaverine and putrescine was gradually increased. More significant differences between inoculated samples and non-inoculated samples were observed at 4 °C than at 30 °C, indicating that the *P. fluorescens* remained high decarboxylase activity at low temperature.

Cadaverine and putrescine can be produced by lysine, ornithine and arginine (Jørgensen et al. 2000). At the end of storage, the changes of most amino acids were observed from Table 3 since the formation of biogenic amines which attribute to decarboxylation of amino acids. Lysine could be transformed to cadaverine by *P. fluorescens* (De las Rivas et al. 2006). Compared to the control samples, a marked decline in lysine in the inoculated samples was observed. Arginine transformed into ornithine by action of enzymes (Coffino 2001). According to research (De las Rivas et al. 2006), the target gene of ornithine decarboxylase was found in *P. fluorescens* R2f. The rapid decarboxylation of putrescine in inoculated samples may have occurred.

In conclusion, the growth of *P. fluorescens* may led to the accumulation of spoilage products including TVB-N, cadaverine and putrescine and the degradation of muscle protein and amino acids. Higher spoilage activity and more consumption of amino acids in salmon were observed when *P. fluorescens* cultured at low temperatures. The results highlighted that *P. fluorescens* may play an important role during cold storage of salmon. To understand the

Table 3 Contents of total amino acid in salmon fillets at 30, 10 and 4 °C

Amino acid	Total amino acid (mg/g)						
	0 h	Control			Inoculated sample		
		30 °C	10 °C	4 °C	30 °C	10 °C	4 °C
Asparaginic	19.15	17.28	13.99	16.04	15.45	16.95	11.44
Threonine	13.64	12.77	9.22	9.80	13.24	10.30	11.62
Serine	9.47	8.25	8.49	7.73	9.34	8.91	9.00
Glutamic	31.65	31.34	28.92	26.25	23.42	20.92	25.25
Glycine	10.65	8.56	8.54	10.03	10.19	9.18	9.58
Alanine	14.23	13.44	11.95	11.74	13.24	12.24	10.94
Cystine	10.10	8.40	7.72	7.46	3.42	6.06	5.63
Valine	8.83	9.27	9.28	8.39	10.44	9.41	10.23
Methionine	8.86	7.98	7.34	8.08	7.18	7.34	7.98
Isoleucine	7.64	8.23	8.35	7.32	9.36	8.29	9.20
Leucine	16.45	13.95	15.46	15.72	12.63	15.95	16.42
Tyrisine	9.65	8.86	7.18	9.25	8.41	9.41	8.02
Phenylalanine	6.41	6.54	6.88	6.06	7.17	7.00	7.47
Lysine	19.60	16.45	14.39	15.32	13.19	12.88	12.32
Histidine	7.02	5.94	6.85	6.68	6.02.	8.12	5.27
Arginine	15.81	12.73	11.58	13.43	11.54	13.18	9.43
Proline	4.87	4.02	4.53	4.64	2.45	3.83	3.52
Total	214.03	194.01	180.67	183.94	170.67	179.97	173.32

metabolize mechanism for *P. fluorescens* at low temperatures, more investigations are remained to be studied in future.

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