Degradation of histamine by the halotolerant *Staphylococcus carnosus* FS19 isolate obtained from fish sauce

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1. Introduction

Histamine is a biologically active amine compound that is present in various food products due to the bacterial decarboxylation of free histidine. High levels of histamine in foods are undesirable because they may have adverse effects on the health of consumers (Shalaby, 1996). Fish and its fermented products are rich in free amino acids, rendering them vulnerable to bacterial decarboxylase activity; thus, they might contain high levels of biogenic amines, especially histamine. The Food and Drug Administration (FDA) suggested 50 ppm and 500 ppm as the levels of toxic and defect-inducing levels of histamine in fish products, respectively (Lehane & Olley, 2000). Other amines, including tyramine, putrescine, cadaverine and tryptamine, are also present in foods and their significance for the safety and quality of food have been extensively studied. Histamine is an odourless compound that is undetectable even by panellists trained in organoleptic analysis (Tapingkae, Parkin, Tanasupawat et al., 2010). Histamine and other biogenic amines are also heat-stable, and even the autoclaving temperature does not destroy them (Luten et al., 1992). Various approaches, such as modified atmosphere packaging, irradiation, high hydrostatic pressure, food additives and preservatives, as well as the use of negative-amine producer starter cultures have been tested to control the accumulation of biogenic amines in food products (Latorre-Moratalla et al., 2010; Nails, Flint, Fletcher, Bremer, & Meerdink, 2010). However, the mechanism underlying those methods for controlling the content of amines is mainly the inhibition of the growth of amine-producing bacteria and amino acid decarboxylase activities. Irradiation is an effective process for degrading histamine molecules, but it has the potential health hazardous of generating free radical compounds as the major drawback (Kim et al., 2004; Kim, Kim, Ahn, Park, & Byun, 2005). Therefore, efforts to degrading biogenic amines in foods without posing detrimental effects on safety and quality remain an important issue.

Histamine is physiologically degraded through the oxidative deamination process, catalysed by either histamine oxidase or histamine dehydrogenase. Histamine oxidase catalyses the conversion of histamine, in the presence of water and oxygen, to imidazole acetaldehyde, ammonia and hydrogen peroxide.
(Sekiguchi, Makita, Yamamura, & Matsumoto, 2004). Previous studies have demonstrated the presence of histamine oxidase in many bacterial species (Martuscelli, Crudele, Gardini, & Suzuki, 2000; Murooka, Doi, & Harada, 1979; Yamashita, Sakae, Irawata, Sugiono, & Murooka, 1993; Zaman, Bakar, Selamat, & Bakar, 2010). In addition, several bacteria also utilise histamine dehydrogenase to degrade histamine (Hacisalihoğlu, Jongejan, & Duine, 1997). Recently, the application of bacteria possessing histamine-degrading enzyme has become an emerging technology for reducing the histamine concentration in foods, especially fermented products (Mah & Hwang, 2009; Naidu et al., 2010). Nevertheless, certain food products pose restrictions on this activity in terms of bacterial growth and enzyme activity at a low pH, high temperature or high salinity. These restrictions should be eliminated to ensure the efficacy of this activity in foods with a high salt content, such as fish sauce and shrimp sauce. However, only a few available reports have focused on bacteria that exhibit biogenic amine-degrading activity in such extreme environments. For instance, Arthrobacter crystalliopyeotes KAT-B-807, which was isolated from soil, was found to possess a thermophilic histamine oxidase (Sekiguchi et al., 2004), and Natrinema gari, which was isolated from fish sauce, exhibited histamine degradation activity in a high-salt environment (Tapingkae, Parkin, et al., 2010; Tapingkae, Tanasupawat, Parkin, Benjakul, & Visessanguan, 2010). In our previous study, S. carnosus FS19 isolated from fish sauce is known to be a histamine degrader. Therefore, this study is aimed to evaluate the effects of different conditions on the histamine-degradation activity of S. carnosus FS19.

2. Materials and methods

2.1. Preparation of inoculums

S. carnosus FS19 used in this study is isolated from fish sauce samples from north-eastern part of Malaysia. It is exhibited the ability to degrade histamine in considerable strength, as well as putrescine and cadaverine to a lesser strength (Zaman et al., 2010). A loop-full of a tryptic soy agar slant culture of S. carnosus FS19 was inoculated into 10 mL of tryptic soy broth and incubated at 37 °C for 24 h. Five millilitres of this culture was then transferred to 100 mL of tryptic soy broth and incubated at 37 °C for 24 h. The culture was centrifuged at 10,000 × g for 10 min at 4 °C. The cell pellet was washed by centrifugation in sterile 0.05 M phosphate buffer (pH 7.0). The cell suspension was then adjusted to a concentration of 1 × 10^7 cells/mL in sterile 0.05 M phosphate buffer (pH 7.0) and used as an inoculum. To study the effect of the pH value on histamine degradation, the pH of the phosphate buffer was adjusted with the appropriate buffer solution. Sterile distilled water was used instead of phosphate buffer in the study of histamine degradation in the fish sauce samples.

2.2. Effect of pH on the growth and histamine degradation activity of S. carnosus FS19

To study the effect of pH on the growth of S. carnosus FS19, 5 mL of the inoculums was added to 45 mL of tryptic soy broth with the pH adjusted to 4, 5, 6, 7, 8, 9 or 10 using 1 M HCl and 1 M NaOH. The inoculated broth was incubated at 37 °C for 24 h with shaking at 150 rpm (Infors, Bottmingen, Switzerland). The cells present on plate count agar (supplemented with 3% NaCl w/v) after growth at 37 °C for 48 h were counted, and the number of cells was expressed as the log of the CFU/mL.

The effect of pH on histamine degradation by S. carnosus FS19 was investigated by adding 2 mL of the inoculum to 18 mL of sterile buffer medium. It is including 0.05 M acetate buffer (acetic acid and sodium acetate) for pH 4–5, 0.05 M phosphate buffer (mono and dibasic sodium phosphate) for pH 6–7 and 0.05 M Tris buffer for pH 8–9. Each buffer was supplemented with histamine dihydrochloride to 100 ppm and NaCl to 3% (w/v) in the final volume. The mixed solutions were then incubated (Infors, Switzerland) at 35 °C for 24 h with shaking at 150 rpm. Samples (5 mL) were taken and added to an equal amount of 1 M HCl (Merck, Darmstadt, Germany). These mixtures were boiled for 10 min and centrifuged (Sigma 3-18K, Sartorius, Goettingen, Germany) at 9000 × g for 10 min. The supernatant was frozen at −20 °C prior to the analysis of biogenic amines.

2.3. Effect of salt concentration on the growth and histamine degradation activity of S. carnosus FS19

To study the effect of the salt concentration on the growth of S. carnosus FS19, 5 mL of inoculum was added to 45 mL of tryptic soy broth that was supplemented with 0%, 3%, 6%, 9%, 12%, 15% or 18% NaCl. The inoculated broth was incubated at 37 °C for 24 h with shaking at 150 rpm. The cells present on plate count agar (supplemented with 3% NaCl w/v) after growth at 37 °C for 48 h were counted, and the number of cells was expressed as the log of the CFU/mL.

The effect of the salt concentration on histamine degradation by S. carnosus FS19 was studied by adding 2 mL of inoculum to 18 mL of sterile 0.05 M phosphate buffer (pH 7.0) medium that was supplemented with 100 ppm of histamine dihydrochloride. Sodium chloride was added to achieve final concentrations of 0%, 3%, 6%, 9%, 12%, 15% or 18% (w/v). The mixtures were then incubated at 35 °C for 24 h with shaking at 150 rpm. Samples (5 mL) were taken and added to an equal amount of 1 M HCl. These mixtures were boiled for 10 min and centrifuged at 9000 × g for 10 min. The supernatants were frozen at −20 °C prior to the analysis of biogenic amines.

2.4. Effect of temperature on the growth and histamine degradation activity of S. carnosus FS19

To study the effect of temperature on the growth of S. carnosus FS19, 5 mL of inoculum was added to 45 mL of tryptic soy broth that was supplemented with 3% salt. The inoculated broth was incubated at 25, 30, 35, 40, 45 or 50 °C for 24 h with shaking at 150 rpm. The cells present on plate count agar (supplemented with 3% NaCl w/v) after growth at 37 °C for 48 h were counted, and the number of cells was expressed as the log of the CFU/mL.

The effect of temperature on histamine degradation by S. carnosus FS19 was investigated by adding 2 mL of inoculum to 18 mL of sterile 0.05 M phosphate buffer (pH 7.0) medium supplemented with 100 ppm of histamine dihydrochloride and 3% NaCl (w/v) in the final volume. The mixed solutions were then incubated at 30, 35, 40, 45 or 50 °C for 24 h with shaking at 150 rpm. Samples (5 mL) were taken and added to an equal amount of 1 M HCl. The mixtures were then boiled for 10 min and centrifuged at 9000 × g for 10 min. The supernatants were frozen at −20 °C prior to the analysis of biogenic amines.

2.5. Degradation of histamine in fish sauce samples by S. carnosus FS19

Five millilitres of inoculum was added to a 100 mL Erlenmeyer flask containing 45 mL of fish sauce samples obtained from a market in Serdang, Selangor, Malaysia. All of fish sauce samples were made from anchovies and contain different percentage of salt content (Sample A: 18%, B: 28%, C: 30% and D: 22%). For the control samples, instead of starter culture, 5 mL of sterile distilled water was added to 45 mL of fish sauce. The mixtures were then
incubated (Infors, Bottmingen, Switzerland) at 37 °C for 24 h with shaking at 150 rpm. The incubation time was prolonged to five days for a separate batch to study the time course of histamine degradation. The samples were subjected to an extraction procedure prior to the analysis of biogenic amines.

2.6. Determination of pH and salt concentration

The pH value of the fish sauce samples was determined directly using an electronic pH meter (Mettler Toledo 8603, Switzerland). The salt concentration of a tenfold dilution of each sample was determined using a salt meter (Atago ES-421, Japan).

2.7. Determination of biogenic amines

Determination of the biogenic amine contents was performed by high-performance liquid chromatography (HPLC) according to the method proposed by Hwang, Cahng, Shiu, and Chai (1997) and modified by Ozogul, Taylor, Quantick, and Ozogul (2002). Briefly, each fish sauce sample was transferred to a 50 mL centrifuge tube and homogenised with 20 mL of 6% trichloroacetic acid for 3 min. The homogenates were centrifuged (Sigma 3–18K, Sartorius, Germany) at 10,000 × g at 4 °C for 10 min and filtered through Whatman paper No.1. The filtrates were then transferred to a volumetric flask, and 6% trichloroacetic acid was added to a final volume of 50 mL. A series of mixed standard amine solutions of histamine dihydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride and tyramine hydrochloride was prepared to obtain the standard curve for each of these amines. To each 1 mL of a standard amine solution and each extracted sample, 1 mL of 2 M sodium hydroxide was added, followed by 10 µL of benzoyl chloride. The solutions were mixed using a vortex mixer and then incubated at 30 °C for 40 min. The benzoylation reaction was stopped by adding 2 mL of a saturated sodium chloride solution and the solution was extracted with 3 mL of diethyl ether. After centrifugation, the upper layer was transferred to a tube and evaporated to dryness in a stream of nitrogen. The residue was then dissolved in 1 mL of acetonitrile, and 20 µL aliquots were injected into an HPLC apparatus. The HPLC analysis was performed with a Waters 600 controller and pump, a Waters in-line degasser and a Waters 2996 photodiode array detector (set at 254 nm). The chromatographic column used was a Sunfire™ C18, 5 µm, 150 × 4.0 mm (Waters, Milford, USA). Water and acetonitrile were used for gradient elution with a flow rate of 1 mL/min.

2.8. Statistical analysis

All of the experiments were conducted with three replications. The data were subjected to an analysis of variance (ANOVA) and are reported as the mean values ± the standard deviation. The mean comparison was performed using Duncan’s Multiple Range Test (DMRT). The significant difference was set at P < 0.05. All of the statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 16.0 for windows (SPSS Inc., Chicago, Illinois).

3. Results and discussion

3.1. Effect of pH on the growth and histamine degradation activity of S. carnosus FS19

*S. carnosus* FS19 exhibited tolerance to a broad range of pH values, but its optimal growth was observed at pH 8 (Fig. 1A). A decrease in the viable cell count of approximately 5 log cycles (compared to that at the optimal pH) was observed in medium with pH 4. The pH value also influenced the histamine degradation activity of *S. carnosus* FS19. The results suggested that the greatest histamine degradation occurred within the pH range of 5–7 (Fig. 1B). The highest histamine degradation activity was observed at pH 6, degrading up to 32.5% of the histamine with 24 h of incubation at 37 °C. However, more acidic and more alkaline conditions markedly inhibited the degradation activity of this isolate. *S. carnosus* FS19 degraded only 5.3% and 1.7% of the histamine in medium at pH 4 and pH 9, respectively. Similar results have been reported for histamine degradation by *Natrinema gari* BCC 24369, which was isolated from fish sauce (Tapingkae, Parkin, et al., 2010). This halophilic archaeon exhibited optimal degradation activity at pH 6.5–7.5, but it loses 90% and 30% of this activity at pH 4 and pH 9, respectively. In contrast, the histamine oxidase activity of *Arthrobacter crystall pocites* KAIT-B-007 was found to be optimal at pH 9 (Sekiguchi et al., 2004). The histamine degradation activity of this bacterium was found to be catalysed by an endogenous enzyme (Murooka et al., 1979). Hence, the activity occurs within the cell, where the pH is constant while the cell remains intact and viable. The fact that the pH of the medium influenced the histamine degradation activity may be attributable to the loss of cell viability in acidic and alkaline conditions. Thus, the profile for the histamine degradation activity of *S. carnosus* FS19 is consistent with its growth profile across the range of various pH values.
3.2. Effect of salt on the growth and histamine degradation activity of \textit{S. carnosus} FS19

\textit{S. carnosus} FS19 grew in the absence and presence of a high level of salt (Fig. 2A), with optimal growth in 9\% salt (9.35 log CFU/mL). The number of cells obtained was significantly reduced to 5.96 log CFU/mL in medium containing 18\% salt. Probst et al. (1998) also found that some strains of \textit{S. carnosus} isolated from fermented fish and shrimp tolerated 15\% salt, and some could even grow in medium containing as much as 20\% salt. Bacteria will lose their turgor pressure when cultivated in a high-salt medium, leading to physiological and metabolic disturbances (Liu, Asmundson, Gopal, Holland, & Crow, 1998). Regulation of the osmotic pressure between the inside and outside of a cell is the mechanism that allows some bacteria to overcome the effects of a high-salt environment (Kashket, 1987). This trait is found only in halotolerant and halophilic bacteria that can grow in solutions containing as much as 5\% salt and even more than 12\% salt (Frazier & Westhoff, 1988). Thus, \textit{S. carnosus} FS19 may be considered a halotolerant or halophilic bacterium.

A high rate of histamine degradation activity by \textit{S. carnosus} FS19 occurred in the presence of salt. Nevertheless, this activity was also observed, although at a much lower rate, in the absence of salt. The highest level of activity occurred in the presence of 9\% salt, in which up to 39.1\% of the histamine was degraded (Fig. 2B). Sinsuwan, Montriwong, Rodtong, and Yongsawatdigul (2010) also found that histamine degradation by \textit{Brevibacillus} sp. SK35 isolated from fish sauce was optimal in medium containing 10\% salt. The optimal activity of the histamine-degrading enzymes of \textit{Natrinema gari} BCC 24369 and \textit{Natrinema gari} HDS3-1 was observed in the presence of 4–5 M sodium chloride (Tapingkae, Parkin, et al., 2010; Tapingkae, Tanasupawat, et al., 2010). Moreover, \textit{S. carnosus} FS19 exhibited the ability to degrade 22.1\% of histamine in the presence of 18\% salt. This might be explained by its adaptability to high salinity, which influences its enzymatic properties. In general, many salt-loving (halophilic) enzymes require the presence of sodium chloride or potassium chloride within the range of 1–4 M for their optimal activity and stability (Mevarech, Frolov, & Gloss, 2000).

3.3. Effect of temperature on the growth and histamine degradation activity of \textit{S. carnosus} FS19

As shown at Fig. 3A, \textit{S. carnosus} grew well at temperatures ranging from 30 °C to 40 °C. The highest viable count (9.01 log CFU/mL) of this bacterium was observed at 35 °C. The growth was almost completely inhibited at temperatures above 45 °C. In agreement with these results, Probst et al. (1998) reported that \textit{S. carnosus} could grow at 42 °C but could not grow at 45 °C.

The histamine degradation activity of \textit{S. carnosus} FS19 is temperature dependent. The highest activity occurred at 40 °C, and at

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**Fig. 2.** Effect of salt concentration on the growth (A) and histamine degrading activity (B) of \textit{Staphylococcus carnosus} FS19. Error bars represent standard deviation of three replicates. Bars labelled with different letters are significantly different ($P < 0.05$).

**Fig. 3.** Effect of temperature on the growth (A) and histamine degrading activity (B) of \textit{Staphylococcus carnosus} FS19. Error bars represent standard deviation of three replicates. Bars labelled with different letters are significantly different ($P < 0.05$).
this temperature, 23.5% of the histamine was degraded after incubation for 24 h (Fig. 3B). This activity was markedly inhibited by temperatures higher than 40°C. When the incubation temperature was increased to 45°C and 50°C, only 6.1% and 3.3% of the histamine was degraded in the cultures, respectively. In accordance with this result, Sinsuwan et al. (2010) found that histamine is optimally degraded by Brevibacillus sp. SK35 at 35°C. The histamine-degrading archaeon Natrinema gari BCC 24369 exhibited its optimal degradation activity at 40–50°C in a buffer system when applied as free or immobilised cells, but the activity rapidly decreased at temperatures higher than 55°C (Tapingkae, Parkin, et al., 2010). Enzyme activity is inhibited by high temperature due to protein denaturation unless the enzyme is thermostable. Sekiguchi et al. (2004) observed that the histamine oxidase from Arthrobacter crystallopoietes KAIT-B-007 is thermostable because it retained its full activity at 60°C. Most of the growth-associated enzymes are known to exhibit the optimal activity at the same temperature as optimal growth occurs. The fact that the growth of S. carnosus FS19 is retarded at 50°C might explain why its histamine degradation activity is inhibited at that same temperature.

3.4. Degradation of histamine in fish sauce samples by S. carnosus FS19

The ability of S. carnosus FS19 to degrade histamine was expressed in several fish sauce products (Fig. 4A). Fish sauce samples have different salt contents, as shown in Table 1. S. carnosus FS19 also exhibited the ability to degrade approximately 14.6% and 11.4% of the putrescine and cadaverine contents (Fig. 4B and C), respectively, of sample A. Nevertheless, tyramine degradation was not observed in any of the fish sauce samples (Fig. 4D). It seems that the degradation activity was restricted by salt concentration in the samples. The histamine concentration was reduced by approximately 15.1% and 13.8% in fish sauce A (18% salt) and D (21% salt), respectively. Nevertheless, histamine degradation was not observed in samples B and C that contain 29% salt. This might be due to the growth inhibition of this bacterium at very high salinity. Moreover, the ability of bacteria to degrade histamine in pure medium and in food samples is often different. The time course of histamine degradation was investigated in fish sauce A that contains a high level of histamine. S. carnosus FS19 degraded histamine continuously throughout the incubation period (Fig. 5). Nevertheless, the histamine level increased from the third to the fifth day of incubation, which might be due amine formation by histamine-producing bacteria that were likely present in the samples. This would be verified by observing an increase in the concentration of

**Table 1**

<table>
<thead>
<tr>
<th>Fish sauce</th>
<th>S. carnosus FS19</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4.97 ± 0.03</td>
<td>5.16 ± 0.01</td>
</tr>
<tr>
<td>B</td>
<td>5.64 ± 0.01</td>
<td>5.61 ± 0.01</td>
</tr>
<tr>
<td>C</td>
<td>4.76 ± 0.01</td>
<td>4.81 ± 0.06</td>
</tr>
<tr>
<td>D</td>
<td>4.87 ± 0.03</td>
<td>4.82 ± 0.04</td>
</tr>
<tr>
<td>Salt concentration (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>18.50 ± 0.20</td>
<td>18.57 ± 0.12</td>
</tr>
<tr>
<td>B</td>
<td>28.17 ± 0.55</td>
<td>28.07 ± 0.21</td>
</tr>
<tr>
<td>C</td>
<td>29.87 ± 0.15</td>
<td>29.73 ± 0.25</td>
</tr>
<tr>
<td>D</td>
<td>21.93 ± 0.32</td>
<td>21.77 ± 0.21</td>
</tr>
</tbody>
</table>

All values are means ± SD of three replications. Control samples are treated with sterile distilled water instead of the culture.
histamine in the treated and control samples. However, histamine degradation was not observed in the control samples and the histamine concentration slowly increased during the incubation. On the fifth day of incubation, the histamine level was 441.8 and 514.6 ppm in the treated and the control samples, respectively.

*S. carnosus* FS19 also exhibited the ability to degrade putrescine and cadaverine by approximately 14.6% and 11.4% (Fig. 4B and C), respectively in sample A. Degradation of either of these amines was not observed in fish sauce samples with a salt content higher than 18%. This bacterium is known to have a poor ability to degrade putrescine and cadaverine (Zaman et al., 2010). Nevertheless, tyramine degradation was not observed in any of the fish sauce samples (Fig. 4D). This could be due to the absence of tyramine degrading enzymes in the culture. Other possibilities, such as the environmental condition of samples not being suitable for tyramine degrading enzyme activity may also explain this result. Tyramine degrading enzymes have been found in several strains of *Micrococcus varians* and *Brevibacterium linens* (Leuschner, Heidel, & Hammes, 1998). They reported that the tyrosine oxidase activity of *M. varians* LTH 1540 was strongly inhibited below pH 5. As shown at Table 1, most of samples have pH values lower than 5, which may further explain why a tyramine degradation activity was not observed in this study.

4. Conclusion

In conclusion, *S. carnosus* FS19 can be considered a halotolerant histamine-degrading bacteria. In this study, the highest viable count of this bacterium was observed at pH 8, with a salt concentration of 9% and a temperature of 35 °C. *S. carnosus* FS19 exhibited the highest histamine degradation at pH 6, with a salt concentration of 9% and a temperature of 40 °C. This degradation activity is quite remarkable in a medium with a salt level up to 18%. Thus, *S. carnosus* FS19 can potentially be used to reduce the accumulation of histamine during the fermentation of food in high-salt conditions. To understand the effect of environmental factors on histamine degradation, further studies of more factors and with other histamine-degrading bacteria are required.