Occurrence of Biogenic Amines and Amines Degrading Bacteria in Fish Sauce

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Abstract

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The contents of biogenic amines histamine, putrescine, and cadaverine in fish sauce were determined and the bacteria isolated from the samples were evaluated for their amines degradation activity. Five fish sauce samples contained 62.5–393.3 ppm of histamine, 5.6–242.8 ppm of putrescine, and 187.1–704.7 ppm of cadaverine. Thirty three bacterial isolates produced all three amines, seven isolates produced one or two amines, and one isolate did not produce any amine in differential agar media. Since the strains that produced amines were not supposed to degrade them, only eight isolates were further identified and evaluated for their amines degrading capability. *Bacillus amyloliquefaciens* FS-05 and *Staphylococcus carnosus* FS-19 degraded histamine up to 59.9% and 29.1% from its initial concentration, respectively. *Staphylococcus intermedius* FS-20 and *Bacillus subtilis* FS-12 degraded putrescine and cadaverine up to 30.4% and 28.9%, respectively. Most isolates tolerated the salt concentration of up to 15% and temperature of up to 45°C. The current study provided new information on biogenic amines degrading bacteria, isolated from high-salt-content food products. The amines degradation activity of the bacteria is considered as strain rather than species specific.

Keywords: fish sauce; biogenic amines; amines degradation; *Bacillus amyloliquefaciens*; *Staphylococcus carnosus*

Biogenic amines are low molecular weight basic nitrogenous compounds occurring in many foods, mainly due to the amino acids decarboxylation activities of certain microbes (Halasz *et al*. 1994; Křížek & Kalač 1998). Their presence is undesirable because it can result in toxicological effects to consumers such as hypertension, headache, diarrhea, rash, and localised inflammation when ingested in excessive amounts (Halasz *et al*. 1994; Shalaby 1996). Biogenic amines are widely present in food products, especially fermented foods such as fish sauce, cheese, beer, and sauerkraut (Halasz *et al*. 1994; Shalaby 1996). Fish sauce is a popular fermented fish product used as a condiment in Southeast Asia. It is considered an important source of dietary proteins and amino acids. It contains about 20 g/l of nitrogen 80% of which is in the form of amino acids (Sanceda *et al.* 1996). Despite its nutritional value, several authors reported the presence in fish sauce of high levels of biogenic amines predominated by histamine, putrescine, cadaverine, and tyramine (Mah *et al.* 2002; Stute *et al.* 2002; Cho *et al.* 2006; Tsai *et al.* 2006). Tyramine was present at lower levels and was occasionally found in considerable amounts in some samples (Stute *et al*. 2002; Cho *et al*. 2006; Saaid *et al*. 2009). Other amines such as phenylethylamine, spermine, spermidine, and agmatine are considered as trace amines in fish sauce (Mah *et al.* 2002; Stute *et al.* 2002;

Tsai *et al.* 2006). Histamine is considered as the most active amine and is related to almost all food amines poisoning incidences. Furthermore, the occurrence of putrescine and cadaverine can potentiate and enhance the toxicity of histamine (Shalaby 1996). Unfortunately, it is very difficult to remove amines from food products once they are formed, even with the heat treatment such as autoclaving (Luten *et al.* 1992).

Biogenic amines are physiologically degraded through oxidative deamination catalysed by amines oxidase by the following reaction: $R\text{-}CH_2\text{-}NH\text{-}R' + O_2 + H_2O \rightarrow R\text{-}CHO + H_2N\text{-}R' + H_2O_2$ (Murooka *et al.* 1979; Ishizuka *et al.* 1993; Yamashita *et al.* 1993). Monoamine and diamine oxidases are ubiquitous and play an important role in the metabolism of amines in human, plant, and animal cells. Furthermore, these enzymes have also been found in some bacterial strains. Monoamine oxidase was found in some strains of *Enterobacteriaceae* family such as *Klebsiella*, *Enterobacter*, *Eschericia*, *Salmonella*, *Serratia*, and *Proteus* (Murooka *et al.* 1979). Ishizuka *et al.* (1993) reported that *Micrococcus rubens* possesses putrescine oxidase. Some strains of the food fermenting microorganisms such as *Micrococcus* sp. and *Brevibacterium linens* exhibit the ability to degrade histamine and tyramine, while the strains of *Lactobacillus plantarum*, *Lactobacillus sake*, *Lactobacillus pentosus*, and *Pediococcus acidilactici* only degrade histamine (Leuschner *et al.* 1998). Martuscelli *et al.* (2000) also found that some strains of *Staphylococcus xylosus* isolated from artisanal fermented sausages had a remarkable ability to degrade histamine and tyramine. Since the last few years, the potential role of microorganisms possessing amines oxidase activity has become of a particular interest in the prevention or reduction of biogenic amines accumulation in food products, especially fermented food. Leuschner and Hammes (1998) reported that *Micrococcus varians* LTH 1540 decreased a huge amount of tyramine during ripening of fermented sausages. Reduction of histamine and tyramine accumulation in salted and fermented anchovy was reported when *Staphylococcus xylosus* had been applied as the protective culture (Mah & Hwang 2009). The formation of tyramine, putrescine, and cadaverine in sauerkraut was significantly suppressed by the inoculation with *Lactobacillus plantarum* (Kalač *et al.* 2000). However, no literature data are known on the degradation of biogenic amines by bacteria from fish sauce. Hence, the purpose of this research was to isolate bacteria from fish sauce and examine their amino acid decarboxylation activity. The selected isolates were examined for their ability to degrade amines, namely histamine, putrescine, and cadaverine. Biogenic amines content in fish sauce was also determined. The finding of this research was expected to provide some information on amines degrading bacteria originated from high-salt-content products such as fish sauce.

MATERIAL AND METHODS

Fish sauce samples. Five fish sauce samples were obtained from different traditional factories in Tumpat, Kelantan, Malaysia. The samples were transported for analysis in sealed glass bottles at ambient temperature to the Laboratory of Food Safety and Quality, Faculty of Food Science and Technology, Universiti Putra Malaysia. All samples were made from anchovy and were fermented with sea salt for 6 months (2 samples) and 12 months (3 samples) at ambient temperature. The starter culture or proteolytic enzymes were not applied during the samples fermentation. The samples were used for microbiological analysis immediately after their arrival in the laboratory and the rest were stored at 4°C prior to chemical analysis.

Determination of pH value and salt content. The pH value of fish sauce was determined by direct measurement with an electronic pH meter (Mettler Toledo 8603, Schwerzenbach, Switzerland). The salt content of each sample was determined with a salt meter (Atago ES-421, Tokyo, Japan) after ten fold dilution.

Analysis, isolation, and identification of bacteria. The samples (25 ml) were mixed with 225 ml of peptone water containing 0.85% of sodium chloride and then homogenised in stomacher bag (Bagmixer 400, Model L, Interscience, Paris, France) for 2 minutes. Further, ten fold dilutions were made and then 100 µl of each dilution was spread onto agar plates. Aerobic plate count agar and skim milk agar, both supplemented with 3% of sodium chloride, were used to determine total aerobic and proteolytic bacteria count in fish sauce, respectively. The bacterial colonies were counted after the plates incubation at 37°C for 48 hours. The bacterial numbers were then expressed as log colony forming unit (CFU)/ml. To isolate bacteria from fish sauce, each sample (25 ml) was separately added to 225 ml of

trypticase soy broth (Merck, Darmstadt, Germany) supplemented with 3% of sodium chloride in 500 ml flask. The flasks were then shaken (100 rpm) in an incubator (Infors, Bottmingen, Switzerland) at 37°C for 24 hours. The cultures were spread on trypticase soy agar plates supplemented with 3% of sodium chloride and were then incubated at 37°C for 48 hours. The bacterial colonies that developed on the plates were picked based on their colonies appearance and subsequently streaked on fresh trypticase soy agar plates until pure isolates were obtained. The isolates were then screened for their decarboxylase activity toward histidine, ornithine, and lysine (Sigma-Aldrich, St. Louis, USA) in differential medium proposed by Joosten and Northolt (1989). The colour change of the medium surrounding the bacterial colonies from yellow to purple indicated that the particular bacteria could decarboxylase amino acids. Based on the results, the isolates which did not possess the three amino acid decarboxylase activities were selected for the biogenic amines degradation studies. These isolates were identified using Gen III Microlog Identification System (Hayward, USA). The bacterial culture and identification procedure were performed following from the manufacturer's instruction.

Biogenic amines degradation by bacterial isolates. Biogenic amines degradation test was carried out according to the method developed by Leuschner *et al.* (1998). An overnight culture of each isolate was harvested, washed with 0.05M phosphate buffer (pH 7), and the cell pellet was resuspended in 0.05M phosphate buffer supplemented with 100 ppm of histamine, putrescine, and cadaverine (Merck, Darmstadt, Germany). The cell suspension (20 ml) adjusted to a concentration of 10^7 cells/ml was incubated in a 100 ml flask for 24 h at 37°C, under shaking at 150 rpm. The samples were then taken and added to an equal amount of 1M HCl (Merck, Darmstadt, Germany). The mixture was boiled for 10 minutes and centrifuged (Sigma 3-18K, Sartorius, Gettingen, Germany) at 9000 g for 10 minutes. The supernatant was frozen at –20°C prior to biogenic amines analysis.

Screening for the tolerance to various ranges of the environmental factors. A trypticase soy broth medium was used in this series of studies. An overnight culture of each isolate was used as an inoculum from which cells were obtained by centrifugation and were then resuspended in 10 ml of 0.85% saline. 100 µl of each cell suspension was inoculated into individual bottles. The temperatures tested were 30°C, 37°C, 45°C, 50°C, and 55°C, the concentrations of NaCl were 0%, 5%, 7%, 10%, and 15%, and the pH values tested were 5, 6, 7, 8, and 9. To achieve the desired pH value, the medium was adjusted with 1M HCl or 1M NaOH. The bottles were then incubated for 48 h at a specific temperature or at 37°C for the test of pH and salt concentration. At the end of the incubation, the turbidity in each bottle was considered as the growth indication.

Determination of biogenic amines. Biogenic amines in the samples were determined using HPLC according to the method proposed by Hwang *et al.* (1997) and modified by Ozogul *et al.* (2002). Briefly, the fish sauce samples were transferred to 50 ml centrifuge tubes and homogenised with 20 ml of 6% trichloroacetic acid (Merck, Darmstadt, Germany) for 3 minutes. The homogenates were centrifuged at 10 000 g for 10 min at 4°C) and filtered through Whatman paper No. 1. The filtrates were then placed in a volumetric flask and 6% trichloroacetic acid was added to a final volume of 50 ml. A series of mixed standard amine solutions were also prepared to obtain the standard curve for each amine. To 1 ml of standard amine solution and each sample, 1 ml of 2M sodium hydroxide (Merck, Darmstadt, Germany) was added, followed by 10 µl of benzoyl chloride (Merck, Darmstadt, Germany). The solution was mixed using a vortex mixer and then allowed to stand at 30°C for 40 minutes. The benzoylation was then stopped by adding 2 ml of saturated NaCl solution, and the mixture was extracted with 3 ml of diethyl ether (Merck, Darmstadt, Germany). After centrifugation, the upper layer was transferred into a tube and evaporated to dryness in a stream of nitrogen. The residue was then dissolved in 1 ml of acetonitrile (Merck, Darmstadt, Germany). HPLC determination was performed with a Waters 600 controller and a pump, a Waters in-line degasser, a Waters 2996 photodiode array detector, and Empower 2 Software. A Sunfire^{TM} C_{18}, 5 µm, 150 × 4.0 mm column (Waters, Milford, USA) was used with water and acetonitrile as the mobile phase set for linear gradient at the flow rate of 1 ml/minute. The sample volume injected was 20 µl and it was monitored at the wavelength of 254 nm. The detection limits for histamine, putrescine, and cadaverine were 0.16 ppm, 0.09 ppm, and 0.07 ppm, respectively. The quantification limits for histamine, putrescine, and cadaverine were 0.53 ppm, 0.31 ppm, and 0.24 ppm, respectively.

Statistical analysis. The significance of difference was determined by using the analysis of variance (ANOVA). The comparison of means was carried out with Duncan's multiple range tests (DMRT). Pearson's correlation test was applied to determine the relationship between the variables in the samples. All statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 16.0 for Windows (SPSS Inc., Chicago, USA). The value of $P < 0.05$ was used to indicate significant deviations.

RESULT AND DISCUSSION

The values of the pH, salt content, aerobic plate count (APC), and proteolytic bacteria count in fish sauces are presented in Table 1. The level of pH and salt content in all samples ranged from 4.8% to 5.7%, and 15.6% to 25.7%, respectively. The results were about in the same range with several fish sauces from different countries of origin as reported by Park *et al.* (2001). The differences in the salt content between the samples were mainly due to the different ratios of fish and salt applied by manufacturers at the beginning of fermentation. It was proved that salt content was not much changed in the course of fermentation (Dissaraphong *et al.* 2006). Lopetcharat *et al.* (2001) stated that Malaysian fish sauce producers use the fish to salt ratio of 3–5:1. This ratio complies with The Malaysian Food Act 1983 and Regulation 1985 which require fish sauce to contain not less than 15% of salt (Anonymous 2004). The high salt concentration can prevent the growth of spoilage microorganisms such as *Escherichia* sp., *Serratia* sp., *Pseudomonas* sp., and *Clostridium* sp. in fish sauce (Lopetcharat *et al.* 2001). In general, significant correlations were found between the pH value, salt content, aerobic plate count, proteolytic bacteria count, and biogenic amines content (Table 2). The result showed that the levels of biogenic amines in fish sauces depended on other related variables such as aerobic and proteolytic bacteria counts. A correlation between aerobic bacteria count and amines, namely putrescine, cadaverine, and tyramine, was also found in other products (Valero *et al.* 2005). However, no significant correlation was observed between putrescine and salt ($r = 0.459$, $P = 0.085$) and histamine and pH ($r = 0.501$, $P = 0.057$), in agreement with the correlation previously found in fermented fish products in Taiwan (Tsai *et al.* 2006).

Figure 1 shows the biogenic amines contents in fish sauce samples. Histamine content ranged from 62.5 ppm to 393.3 ppm. Putrescine and cadaverine contents ranged from 5.6 ppm to 242.8 ppm and 187.1 ppm to 704.6 ppm, respectively. It was reported that the highest histamine, putrescine, and cadaverine contents in fish sauce were 1220, 1257, and 1429 ppm, respectively (Zaman *et al.* 2009). Histamine content in all samples exceeded 50 ppm, the defect level imposed by the US FDA. However, none of the samples exceeded the level of 500 ppm, that is the amount which could be hazardous for the consumer health. Fish sauce B had a higher content of histamine as compared to all other samples. This could be attributed to the higher contents of aerobic and proteolytic bacteria in the particular sample. Biogenic amines accumulation in fish products results mainly from decarboxylation activity of bacteria toward free amino acids (Brink *et al.* 1990). Proteolytic bacteria

Table 1. The value of pH, salt content, aerobic plate count and proteolytic bacteria count of fish sauce

Fish sauce	pH	Salt content (%)	Aerobic plate count	Proteolytic bacteria
			(log CFU/ml)	
A	5.4 ± 0.1^{c}	25.7 ± 0.3^{c}	5.02 ± 0.1^{a}	3.63 ± 0.1^{a}
B	5.7 ± 0.1^{d}	25.3 ± 0.5^{c}	5.53 ± 0.5^{b}	3.97 ± 0.1^{b}
C	5.1 ± 0.1^{b}	18.8 ± 0.3^{b}	4.97 ± 0.1^{a}	3.62 ± 0.2^{a}
D	5.3 ± 0.1^{c}	19.1 ± 0.1^{b}	5.18 ± 0.1^{ab}	3.76 ± 0.1^{a}
E	4.8 ± 0.2^{a}	15.6 ± 0.8^{a}	4.92 ± 0.2^{a}	3.65 ± 0.2^{a}

The numbers represent mean ± SD of three replications. Values followed with same letters within a column are not significantly different in Duncan's multiple range test ($P < 0.05$)

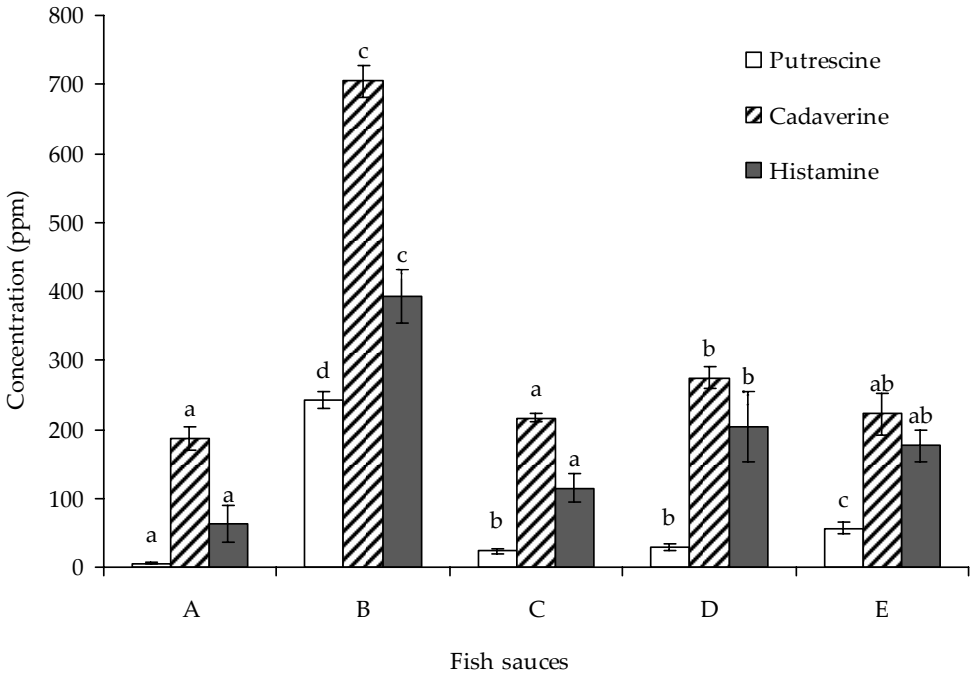

Figure 1. Biogenic amines content in fish sauce samples. Bars represent standard deviation of three replicates. The same coloúr histogram marked with same letters are not significantly different in Duncan's multiple range test ($P < 0.05$)

play an important role in releasing amino acids from the protein tissues offering the substrate for decarboxylation activity by amines producing bacteria. Pearson's correlation analysis of the data revealed a good positive relationship between the aerobic and proteolytic bacteria counts and biogenic amines contents. Putrescine content in all samples was considered to be in a lower range except for sample B (242.8 ppm), particularly when compared to the previous report on Korean fish sauce (Cho *et al.* 2006). This could be due to the low presence of putrescine producing bacteria during the fermentation of the samples. Unlike of putrescine, most of samples have a high content of cadaverine, the level reaching 704.6 ppm in sample B. Cadaverine content normally increases during fish sauce fermentation because more lysine (precursor of cadaverine) is released from the protein complex of fish. Park *et al.* (2001) reported that lysine as well as histidine, aspartate, glutamate and alanine, was dominant in most of fish sauce samples. The presence of bacteria capable to de-

Table 2. Correlation between biogenic amines content, salt, pH value, aerobic plate count (APC) and proteolytic bacteria

	Putrescine	Cadaverine	Histamine	Salt	APC	Proteolytic bacteria	pH
Putrescine	1.000						
Cadaverine	0.981** (0.000)	1.000					
Histamine	0.923** (0.000)	0.923** (0.000)	1.000				
Salt	0.459 (0.085)	0.613* (0.015)	0.571* (0.026)	1.000			
APC	0.715** (0.003)	0.764** (0.001)	0.720** (0.002)	0.640* (0.010)	1.000		
Proteolytic bacteria	0.774** (0.001)	0.833** (0.000)	0.742** (0.002)	0.643** (0.010)	0.610* (0.016)	1.000	
pH	0.586* (0.022)	0.689** (0.004)	0.501 (0.057)	0.755** (0.001)	0.555* (0.032)	0.629* (0.012)	1.000

Pearson correlation *P*-value: *significant at the 0.05 level; **significant at the 0.01 level

Table 3. Amino acids degradation by bacteria isolated from fish sauce

Code and identity of isolates		Amino acids degradation		
		His	Orn	Lys
FS-05	*Bacillus amyloliquefaciens*	–	+	+
FS-12	*Bacillus subtilis*	–	+	+
FS-13	*Bacillus humi*	–	–	+
FS-14	*Bacillus amyloliquefaciens*	–	–	+
FS-19	*Staphylococcus carnosus*	–	–	–
FS-20	*Staphylococcus intermedius*	+	–	–
FS-22	*Staphylococcus condiment*	+	–	–
FS-25	*Staphylococcus carnosus*	–	+	–

His – histidine, histamine precursor; Orn – ornithine, putrescine precursor; Lys – lysine, cadaverine precursor

carboxylase lysine will result in the accumulation of cadaverine in the product. Putrescine and cadaverine are potentiators of histamine toxicity as they inhibit the histamine-metabolising enzyme in the small intestine (Shalaby 1996; Lehane & Olley 2000). Hence, the high content of both amines in the presence of even a small amount of histamine could possibly alter histamine toxicity.

Forty one bacterial isolates were obtained from fish sauce samples. All were tested and found to be gram positive, 33 cocci and 8 rod shaped bacteria. All isolates were qualitatively tested for their amino acid decarboxylase activity toward histidine, ornithine, and lysine. Thirty three isolates exhibited the ability to produce all tested amines in qualitative medium. Two isolates produced two amines, five isolates produced one amine, and one isolate did not produce any amine (Table 3). The ability of bacteria to produce more than one amine had been revealed by many studies. Dapkevicius *et al.* (2000) suggested that attention must be given to biogenic amines production when selecting bacteria for starter culture. Moreover, Leuschner *et al.* (1998) suggested that strains possessing amino acid decarboxylase activity might not degrade biogenic amines. It is quite difficult to find bacteria without any amino acid decarboxylase activity involved in food fermentation, particularly in the products with a high protein content such as fish sauce. Therefore, the isolates which did not produce all the three amines were further screened for their ability to degrade amines and tolerance to several environmental conditions.

Table 3 shows that all isolates had the ability to degrade the amines tested, although with different efficiencies. Histamine is the most active amine and abundant in fish sauce and other fish products. Hence, the isolates that exhibited a high histamine degradation activity were of particular interest. *Bacillus amyloliquefaciens* FS-05 exhibited a significantly higher potential to degrade histamine than other isolates (Table 4). These rod shaped bacteria degrade histamine to about 59.9% of its initial content within 24 h, while the other

Table 4. Biogenic amines degradation by bacteria isolated from fish sauce after incubation in 0.05M phosphate buffer supplemented with 100 ppm of amines for 24 h at 37°C

Bacterial isolates	Histamine		Putrescine		Cadaverine	
	ppm	degradation (%)	ppm	degradation (%)	ppm	degradation (%)
FS-05	40.1 ± 6.8	59.9^{c}	92.5 ± 2.6	7.5^{ab}	73.6 ± 2.7	26.4^{b}
FS-12	65.8 ± 3.3	34.2^{b}	70.3 ± 5.2	29.7 c	71.1 ± 9.6	28.9^{b}
FS-13	67.2 ± 5.7	32.8^{b}	85.2 ±5.5	14.8^{b}	76.1 ± 8.2	23.9^{b}
FS-14	73.4 ± 9.3	26.6^{b}	69.7 ± 6.3	30.0^{c}	77.7 ± 13.9	22.3^{b}
FS-19	70.9 ± 4.3	29.1^{b}	92.1 ± 3.8	7.8^{ab}	91.6 ± 3.6	8.4 a
FS-20	89.1 ± 1.2	10.8^{a}	69.6 ± 5.1	30.4^{c}	71.9 ± 3.8	28.0^{b}
FS-22	72.6 ± 9.4	27.4^{b}	95.6 ± 1.3	4.4^{a}	93.2 ± 2.4	6.8^{a}
FS-25	95.1 ± 3.9	4.9^{a}	86.1 ± 1.2	13.9^{b}	92.6 ± 2.6	7.4^{a}

The numbers represent mean ± SD of three replications. Values followed with same letters within a column are not significantly different in Duncan's multiple range test ($P < 0.05$)

Table 5. Tolerance of the bacterial isolates to ranges of temperature, NaCl concentration, and pH

Environmental factors	Isolates							
	FS-05	FS-12	FS-13	FS-14	FS-19	FS-20	FS-22	FS-25
Temperature (°C), TSB + 5% NaCl, incubated for 48 h								
30	+	+	+	+	+	+	+	+
37	+	+	+	+	+	+	+	+
45	+	+	–	+	+	+	–	+
50	–	+	–	+	–	–	–	–
55	–	–	–	–	–	–	–	–
NaCl concentration (%), TSB, incubated at 37°C for 48 h								
0	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+
15	+	+	-	+	+	+	+	-
pH, TSB + 5% NaCl, incubated at 37 °C for 48 h								
5	+	+	-	-	+	+	+	+
6	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
9	+	+	-	-	+	+	+	+

+ indicate growth; – indicate no growth

cultures have a lower activity in degrading this amine. The *Bacilli* isolated from fish sauce such as *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus megaterium*, and *Bacillus licheniformis* are well known for their protein degradation activity (Fu *et al.* 2008). Little is known about the amine degradation activity of the bacteria belonging to this genus. In agreement with our results, other studies found *Bacillus* sp. LMG 21002 and *Bacillus megaterium* KL-197 isolated from fish sauce to contain histamine degrading enzymes (Kim & Kim 2006). Mah and Hwang (2009) also described histamine degrading enzymes in some strains of *Bacillus coagulans*. Histamine degradation activity was also found in isolates identified as *Staphylococcus*. *Staphylococcus condiment* FS-22 and *Staphylococcus carnosus* FS-19 reduced histamine content to about 27.4% and 29.1%, respectively. The species of *Staphylococcus*, common bacteria isolated from fish sauce, play an important role in the protein degradation and flavour or aroma development (Tanasupawat 1991; Um & Lee 1996; Lopetcharat *et al.* 2001; Fukami *et al.* 2004; Yongsawatdigul *et al.* 2007). Some species are known as amines producing bacteria, while others are known as amine degrading bacteria. Martuscelli *et al.* (2000) observed in their study that strains of *Staphylococcus xylosus* had a high ability to degrade histamine in a buffer system, degrading up to 100% of histamine. In contrast with our findings, Leuschner *et al.* (1998) reported that strains of *Staphylococcus carnosus* did not possess histamine degradation activity. This result suggested that amines degradation activity was not species but strains specific. In addition to histamine degradation activity, we also found that the isolates tested in our study had the ability to degrade putrescine and cadaverine. *Bacillus amyloliquefaciens* FS-14 and *Bacillus subtilis* FS-12 reduced putrescine and cadaverine contents to 30.0% and 28.9%, respectively. *Staphylococcus intermedius* FS-20 degraded 30.4% and 28.0% of putrescine and cadaverine, respectively. This may be of further interest since only little information exists on putrescine and cadaverine degrading bacteria. The activity to degrade putrescine was

also found in other bacteria such as *Micrococcus rubens* (Ishizuka *et al*. 1993).

The isolates could grow in the temperature range between 30°C and 50°C, but none could withstand 55°C (Table 5). Six isolates could grow at 45°C, although only *Bacillus subtilis* FS-12 and *Bacillus amyloliquefaciens* FS-14 could grow at temperatures of up to 50°C. However, most isolates could grow at the temperatures preferable for fish sauce fermentation, generally between 35°C and 45°C (Lopetcharat *et al.* 2001). Most isolates also tolerated pH range suitable for fish sauce (Table 5). Park *et al.* (2001) found in their research that the average pH value of fish sauce from various countries ranged from 4.9 to 6.1. Regarding the salt tolerance, most isolates could grow in media with salt concentration of up to 15% except for *Bacillus humi* FS-13 and *Staphylococcus carnosus* FS-25 (Table 5). Bacteria will lose their turgor pressure when cultivated in a high salt concentration medium which leads to the cell physiology and metabolism disturbance (Liu *et al.* 1998). Some bacteria overcome this effect by regulating the osmotic pressure between the inside and outside of the cell (Kashket 1987). This trait was found in halotolerant and halophilic bacteria which could grow at the salt concentration of up to 5% and more than 12% of salt, respectively (Frazier & Westhoff 1988). Hence, the findings of this research confirmed that most bacteria isolated from fish sauce which exhibited amines degradation activity should belong to halotolerant or halophilic groups. However, amines degradation activity of the bacteria in a buffer system would probably be not the same in complex substances. Mah and Hwang (2009) reported that *Staphylococcus xylosus* No. 0538 showed a lower activity in complex substances as compared to the buffer system. They found that the bacteria reduced 16% of overall biogenic amines when applied as starter culture in salted and fermented anchovy. Therefore, the isolates should be further examined in food systems such as those applying fish sauce fermentation.

CONCLUSION

Histamine content in fish sauce samples exceeded the defect level of 50 ppm designed by US FDA. The product was still considered as probably safe since the consumption of fish sauce mainly as condiment might not lead to an excessive intake of this amine. However, the presence of high levels of amines in fish sauce showed that the product lacked necessary hygiene during fermentation. The findings of this research indicated that, within the bacterial flora of fish sauce, most of bacteria possessed the potential to produce biogenic amines, particularly histamine, putrescine, and cadaverine while others, however, were able to degrade those three amines. The strains of genera *Bacillus* and *Staphylococcus* found in this research were able to degrade one or more of the amines. It may be also concluded that amines degradation activity was rather strain than species specific. The amines oxidase activity as well as safety of these bacteria can be considered as the criteria for the selection of the bacteria used to reduce amine accumulation in fish sauce and other related product.

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