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Original Article

Degradation of histamine by *Bacillus polymyxa* isolated from salted fish products

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ABSTRACT

Histamine is the causative agent of scombroid poisoning, a foodborne chemical hazard. Histamine is degraded by the oxidative deamination activity of certain microorganisms. In this study, eight histamine-degrading bacteria isolated from salted fish products were identified as *Rummeliibacillus stabekisii* (1 isolate), *Agrobacterium tumefaciens* (1 isolate), *Bacillus cereus* (2 isolates), *Bacillus polymyxa* (1 isolate), *Bacillus licheniformis* (1 isolate), *Bacillus amyloliquefaciens* (1 isolate), and *Bacillus subtilis* (1 isolate). Among them, *B. polymyxa* exhibited the highest activity in degrading histamine than the other isolates. The ranges of temperature, pH, and salt concentration for growth and histamine degradation of *B. polymyxa* were 25–37°C, pH 5–9, and 0.5–5% NaCl, respectively. *B. polymyxa* exhibited optimal growth and histamine-degrading activity at 30°C, pH 7, and 0.5% NaCl in histamine broth for 24 hours of incubation. The histamine-degrading isolate, *B. polymyxa*, might be used as a starter culture in inhibiting histamine accumulation during salted fish product fermentation.

1. Introduction

Histamine is the causative agent of scombroid poisoning and a foodborne chemical hazard. Although scombroid poisoning is usually a mild illness with symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin [1], the severity of the illness varies considerably depending on the amounts of histamine ingested and individual's susceptibility to histamine. Histamine is primarily produced in foods through decarboxylation of free histidine by the activity of various species of bacteria. Fish and its fermented products are rich in free amino acids, making them vulnerable to bacterial decarboxylase activity, and thus might contain a high level of biogenic amines, including histamine [2].

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Histamine is heat stable and not detectable through organoleptic analysis, even by trained panelists [3,4]. Various approaches such as modified atmosphere packaging, irradiation, high hydrostatic pressure, using food additives and preservatives, as well as negative amines producer starter cultures have been applied to control the accumulation of biogenic amines in food products [4]. However, the mechanism underlying those methods in controlling amines content is mainly by inhibition of amines producing bacterial growth and amino acids decarboxylase activities. Except for gamma irradiation, no other food processing methods are available for histamine degradation [5,6]. However, irradiation has the potential health hazard of generating free radical compounds, which is a major drawback [5,6]. Therefore, histamine, if present, is difficult to destroy and posts a risk of food intoxication. It is important to degrade histamine in foods to avoid detrimental effects on safety and quality.

Histamine is physiologically degraded through the oxidative deamination process catalyzed by either histamine oxidase or histamine dehydrogenase. Histamine oxidase catalyzes the conversion of histamine, in the presence of water and oxygen, to imidazole acetaldehyde, ammonia, and hydrogen peroxide [7]. Histamine oxidase was found in *Staphylococcus xylosus*, *Staphylococcus carnosus*, *Bacillus amyloliquefaciens*, *Arthrobacter crystallopoietes*, and *Brevibacterium linens* [3,7–9]. In addition, several bacteria also utilize histamine dehydrogenase to catalyze the oxidative deamination of histamine to imidazole acetaldehyde and ammonia [10]. Some bacteria producing histamine dehydrogenase include *Rhizobium* sp., *Nocardioides simplex*, and *Natrinema gari* [4,11–14]. Recently, the application of bacteria possessing histamine degrading enzymes has become an emerging method for reducing histamine concentration in foods, especially in fermented products [4,11]. Nevertheless, certain food products pose restrictions on this application in terms of bacterial growth and enzyme activity at a low pH value, high temperature, or high salinity. However, only a few available reports have focused on bacteria that exhibit biogenic amine-degrading activity in such extreme environments. For instance, *Arthrobacter crystallopoietes* KAIT-B-007 isolated from soil was found to possess thermophilic histamine oxidase [7], and *N. gari* isolated from fish sauce exhibited histamine degradation activity in a high-salt environment [12,13]. However, no literature data are known on the degradation of histamine by bacteria from salted fish products in Taiwan. Hence, the purpose of this research was to isolate histamine-degrading bacteria from salted fish products and to evaluate the influence of environmental conditions such as pH, temperature, and salt concentration on the histamine-degradation activity of *Bacillus polymyxa*.

2. Materials and methods

2.1. Salted fish products

Salted fish product (sardine sample) was purchased from a fishing village store in Taiwan in October 2010. The salted fish product was packed in glass bottles and kept at room temperature in the store before purchase. After purchase, it was immediately transported to the laboratory for use.

2.2. Isolation of histamine-degrading bacteria

A 25-g portion of the salted fish sample was homogenized at high speed for 2 minutes in a sterile blender. Approximately 1.0 g of the homogenate was added to 10 mL of histamine broth (glucose 0.1%, yeast extract 0.2%, NaCl 0.5%, histamine dihydrochloride 0.1%, K_2HPO_4 0.05%; pH 7.0) [14], and cultivated at 30°C for 7 days. Then, 0.1 mL of the culture was spread onto histamine agar plates (2.0% agar in histamine broth) and incubated at 30°C for 7 days. Approximately 50 colonies on the cultured plates were picked and further streaked on trypticase soy agar (TSA) (Difco, Becton Dickinson, Sparks, MD, USA) to obtain pure cultures. The ability of each isolate to degrade histamine was determined by inoculating the isolates in trypticase soy broth (TSB) (Difco) supplemented with 50 ppm histamine dihydrochloride (histamine TSB) and incubating without shaking at 30°C for 24 hours. One milliliter of the culture broth was taken for quantitation of histamine using high-performance liquid chromatography (HPLC) as described in the next paragraph. In this study, only eight bacterial isolates can degrade histamine in histamine TSB broth (Table 1). Among them, *B. polymyxa* D05-1 exhibited the highest activity of histamine degradation (Table 1). Therefore, the effects of temperature, pH, and salt concentration on bacterial growth, histamine dehydrogenase activity, and histamine-degrading capability of *B. polymyxa* D05-1 will be described in the next paragraph.

2.3. Histamine content assay of HPLC

Samples of standard histamine solutions and 1-mL aliquots of the culture broth were derivatized with dansyl chloride according to the previously described method [15]. The dansyl derivatives were filtrated through a 0.45-μm filter, and 20-μL aliquots were used for HPLC injection.

The content of histamine in the culture broth of the isolates was determined using HPLC (Hitachi, Tokyo, Japan) consisting of a Model L-7100 pump, a Rheodyne Model 7125 syringe loading sample injector, a Model L-4000 UV–Vis detector (set at 254 nm), and a Model D-2500 Chromato-integrator. A LiChrospher 100 RP-18 reverse-phase column (5 um, 125 mm × 4.6 mm; E. Merck, Damstadt, Germany) was used for chromatographic separation. The gradient elution program began using 50:50 (v/v) acetonitrile:water at a flow rate of 1.0 mL/min for 19 minutes, followed by a linear increase to 90:10 acetonitrile:water (1.0 mL/min) during the next 1.0 minutes. Finally, the acetonitrile:water mix was decreased to 50:50 (1.0 mL/min) for 10 minutes.

2.4. Identification of histamine-degrading isolates

The histamine-degrading isolates were identified on the basis of morphology, Gram stain, endospore stain, and catalase and oxidase reaction. The identity of histamine-degrading isolates was further confirmed by amplifying and sequencing approximately 1400 bp of 16S ribosomal DNA (rDNA) for bacteria using the primers UNI-L (5′-AGAGTTTGATCATGGCTCAG-3′) and

Table 1 – Identification of histamine-degrading bacteria isolated from salted fish products in Taiwan and histamine degradation by the isolates in trypticase soy broth supplemented with 50 ppm histamine after incubation at 30°C for 24 hours.

Strain	Organism identified	Percentage identity (%)	Genbank accession number	Histamine residual (ppm)[a]	Degradation percentage (%)
A03-1	*Rummeliibacillus stabekisii*	100	AB271737.1	17 ± 0.7 C	66
B03-1	*Agrobacterium tumefaciens*	100	CP002688.1	16 ± 2.1 C	68
C02-1	*Bacillus cereus*	100	HQ438289.1	26 ± 1.4 D	48
D05-1	*Bacillus polymyxa*	99	JF513085.1	0.01 ± 0.01 A	100
E01-1	*Bacillus cereus*	100	HQ438289.1	24 ± 1.4 D	52
F01-1	*Bacillus licheniformis*	100	JF682389.1	13 ± 2.1 B	74
G02-1	*Bacillus amyloliquefaciens*	100	JF697198.1	17 ± 0.7 C	66
N7-2	*Bacillus subtilis*	100	HQ438293.1	13 ± 0.1 B	74

[a] The numbers represent the mean ± standard deviation of three determinations. Values followed by different letters in the same column are significantly different ($p < 0.05$).

UNI-R (5′-GTGTGACGGGCGGTGTGTAC-3′) [16,17]. Bacterial cells were cultured overnight in 2 mL of TSB at 30°C and then centrifuged at 5000*g* for 10 minutes. The cell pellet was washed and resuspended in 0.5 mL of TE-buffer (10 mM Tris–HCl; 1 mM EDTA; pH 8.0), and then lysed using 20% sodium dodecyl sulfate. After the solution was boiled for 20 minutes and the cellular debris was discarded following centrifugation at 13,000*g* for 3 minutes, the total DNA in the supernatant was precipitated with 70% ethanol and used as template DNA for polymerase chain reaction (PCR). PCR amplification was performed in a 20-μL reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 20 pmol of each primer, a 0.2 mM concentration for each of the four deoxynucleotide triphosphates, 0.5 U of Taq DNA polymerase (Applied Biosystems, Foster City, CA, USA), and template DNA (10 ng). Amplifications were carried out for 35 cycles (94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 60 seconds) in a GeneAmp PCR 2400 Thermal Cycler (Applied Biosystems) with an initial denaturation at 94°C for 4 minutes and a final extension at 72°C for 7 minutes [16,17]. Ten microliters of each PCR product were loaded into a well of 1.5% agarose gel staining with ethidium bromide after electrophoresis. Amplicons were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) eluted in Tris–HCl (10 mM, pH 8.5) prior to sequencing. The amplified DNA was directly sequenced using the ABI TaqDye Deoxy Terminator Cycle sequencing kit and ABI Model 377 automated DNA sequencer (Applied Biosystems). The sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) [National Center for Biotechnology Information (NCBI), Bethesda, MD] for identification of histamine-degrading bacteria.

2.5. *Effect of temperature on the growth and histamine degradation activity of B. polymyxa D05-1*

The effect of temperature on the growth and histamine degradation activity of *B. polymyxa* D05-1 was determined in 50 mL of histamine broth (0.5% NaCl and pH 7.0) incubating at 4°C, 15°C, 25°C, 30°C, 37°C, and 45°C, under shaking at 150 rpm. One hundred microliters of the 20-hour-old bacterial cultures in 5 mL of histamine broth at 30°C were inoculated into fresh histamine broth (50 mL) to obtain an initial concentration of approximately 6.0 log colony-forming units (CFU)/mL. Then, the inoculated histamine broths were incubated at different temperatures. Bacterial count, histamine degrading capability, and histamine dehydrogenase activity in the histamine broth were determined after incubation for 0, 6, 12, 18, 24, 36, and 48 hours.

2.6. *Effect of pH on the growth and histamine degradation activity of B. polymyxa D05-1*

The effect of pH on the growth and histamine degradation activity of *B. polymyxa* D05-1 was determined in 50 ml of histamine broth (0.5% NaCl) with the pH adjusted to 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 using 1M HCl and 1M NaOH, under shaking at 150 rpm. One hundred microliters of the 20-hour-old bacterial cultures in 5 mL of histamine broth at 30°C were inoculated into above histamine broth to obtain an initial concentration of approximately 6.0 log CFU/mL. Bacterial count, histamine degrading capability, and histamine dehydrogenase activity in the histamine broth were determined after incubation at 30°C for 24 hours.

2.7. *Effect of salt concentration on the growth and histamine degradation activity of B. polymyxa D05-1*

The effect of salt concentration on the growth and histamine degradation activity of *B. polymyxa* D05-1 was determined in 50 mL of histamine broth (pH 7.0) supplemented with 0.5%, 5%, 10%, 15%, and 20% NaCl, under shaking at 150 rpm. One hundred microliters of the 20-hour-old bacterial cultures in 5 mL of histamine broth at 30°C were inoculated into the above histamine broth to obtain an initial concentration of approximately 6.0 log CFU/mL. Bacterial count, histamine degrading capability, and histamine dehydrogenase activity in the histamine broth were determined after incubation at 30°C for 24 hours.

2.8. *Bacterial count, histamine-degrading capability, and histamine dehydrogenase activity assay*

The bacterial count was determined by plate count assay. The cultured histamine broths were serially diluted with a sterile phosphate buffer (1:9), and 1.0-mL aliquots of the cultured histamine broth, or dilutes were poured onto Petri dishes (9 cm in diameter). Then, 15–20 mL of TSA (Difco) containing

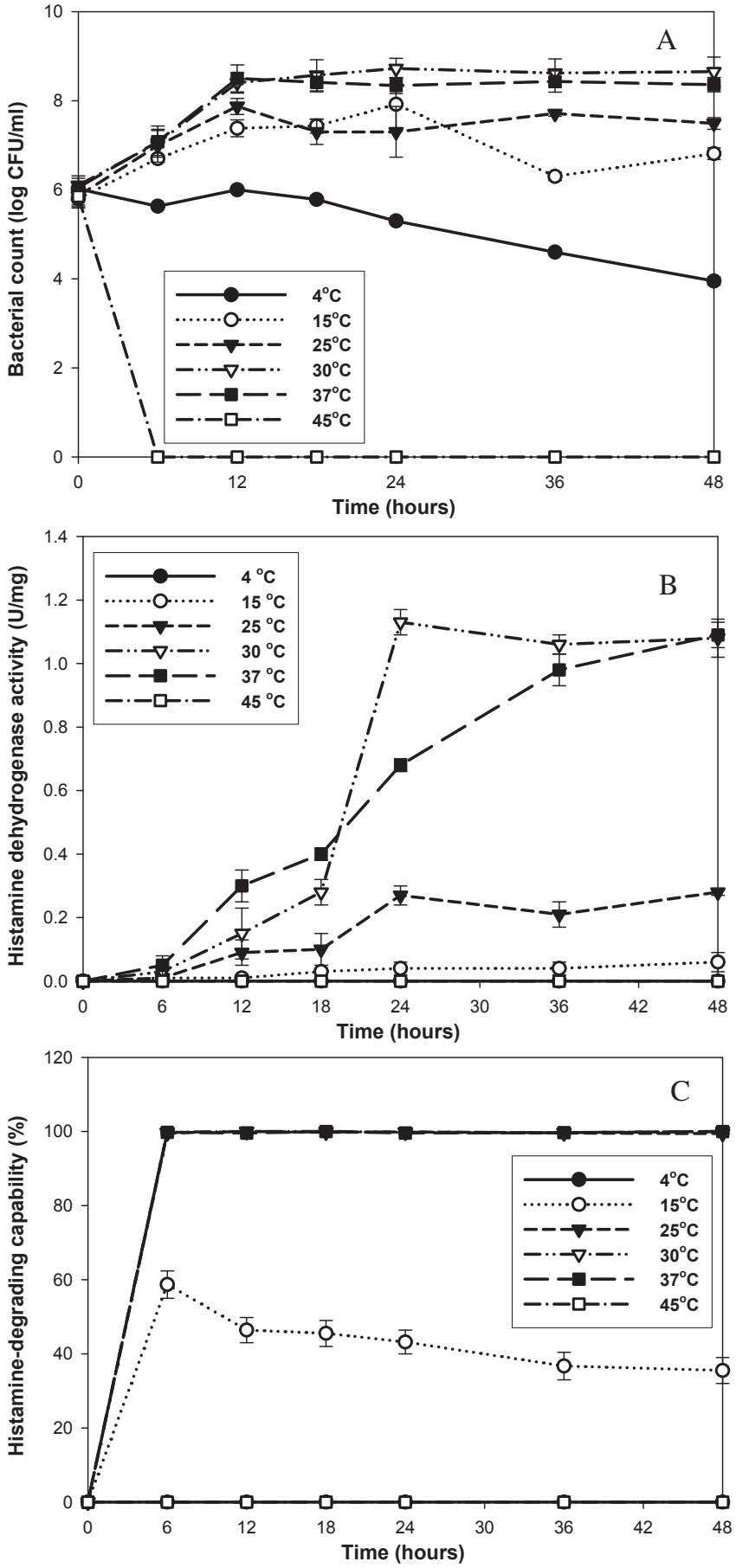

Fig. 1 – Effect of temperature on (A) growth, (B) histamine dehydrogenase activity, and (C) histamine-degrading capability of *Bacillus polymyxa* D05-1. Each value represents the mean ± standard deviation in triplicate.

0.5% NaCl at 45–50°C was added and gently mixed. The poured plates were allowed solidify under a biological clean bench. Bacterial colonies were counted after the plates were incubated at 30°C for 48 hours, and bacterial numbers were expressed as $\log_{10}$ CFU/g.

The histamine-degrading capability of each bacterial isolate was determined by measuring the reduction of histamine in culture media after incubation. One milliliter of the culture broth was taken for quantitation of histamine using HPLC as previously described. The histamine-degrading capability was expressed as the percentage degradation of histamine in the cultured histamine broth.

The histamine dehydrogenase activity was measured spectrophotometrically according to Bakke et al [18] with minor modifications. Cells were harvested from the cultured histamine broths by centrifugation at 10,000*g* for 20 minutes at 4°C, washed with 25 mL of 0.1 M phosphate buffer (pH 7.0), and suspended in 15 mL of the same buffer. The cells suspension was sonicated for 20 seconds, followed by a 40-second rest interval with a total 2-minute sonication using a Sonicator S-4000 (Sonics and Materials Inc., Newtown, CT, USA). The cells and debris were removed by centrifugation at 10,000*g* for 10 minutes at 4°C and discarded. The suspension sample was diluted with 50 mM glycine-NaOH buffer (pH 9.0), and 0.1 mL of the dilution was added to the assay mixture. The assay mixture (2.9 mL) of 83 mM glycine-NaOH buffer (pH 9.0), 0.32 mM histamine dihydrochloride, 10 μM 1-methoxy-5-methylphenazinium methylsulfate (1-methoxy PMS), and 100 μM 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8) was preincubated at 37°C for 5 minutes. The increase of absorbance in the mixture was measured at 460 nm using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol 2-(4-imidazolyl) acetaldehyde per minute under the aforementioned assay conditions. An extinction coefficient of 36 cm^2/μmol of WST-8 was used for the calculation. The protein concentration was determined using a modified Lowry method with a DC™ protein assay kit (Bio-Rad, Hercules, CA, USA) with bovine plasma γ-globulin as the standard protein.

2.9. Statistical analysis

The experiments were conducted using three replications and reported as the mean ± standard deviation. Results were analyzed using analysis of variance and Duncan multiple range test. The significant difference was established at a value of $p < 0.05$. All statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 9.0 for windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Isolation and identification of histamine-degrading bacteria

Table 1 lists the identity of eight histamine-degrading bacteria, determined by 16S rDNA sequences, following comparison to reference strains, using the NCBI database analysis. The PCR amplicon from strain A03-1 had a 100% homology with *Rummeliibacillus stabekisii*, whereas that from strain B03-1 aligned with *Agrobacterium tumefaciens* at 100%. The PCR amplicon from strains C02-1 and E01-1 had a 100% homology with *Bacillus cereus*, whereas that from strain D05-1 had a homology with *B. polymyxa* at 99%. The PCR amplicon from strain F01-1 had a 100% homology with *Bacillus licheniformis*, whereas those from strain G02-1 had a homology with *B. amyloliquefaciens* at 100%. The PCR amplicon from strain N7-2 had a 100% homology with *Bacillus subtilis* (Table 1). All of them were able to degrade histamine in TSB medium supplemented with 50 ppm histamine and reduced levels of histamine in the medium from 0.01 ppm to 26 ppm (52–100% degradation) after incubation (Table 1). Among them, *B. polymyxa* D05 was able to degrade histamine up to 100% in histamine TSB broth.

In this study, six of eight histamine-degrading bacteria (75%) from salted fish samples belonged to *Bacillus* spp., including: *B. cereus* (2 isolates), *B. polymyxa* (1 isolate), *B. licheniformis* (1 isolate), *B. amyloliquefaciens* (1 isolate), and *B. subtilis* (1 isolate). Similarly, *Bacillus* sp. and *Bacillus megaterium* isolated from fish sauce contain histamine degrading enzymes [19]. Mah and Hwang [11] also described histamine degrading enzymes in some strains of *Bacillus coagulans*. Recently, Zaman et al [3] demonstrated that *Bacillus* spp. such as: *B. amyloliquefaciens*, *B. subtilis*, and *B. himi*, isolated from fish sauce degraded 26.6–59.9% of histamine in histamine broth. Although *R. stabekisii* and *A. tumefaciens* were never identified as histamine-formers, they accounted for 25% (2/8) of the total histamine-degrading isolates in this study. Specifically, both histamine-degrading isolates degraded 66% and 68% of histamine in histamine TSB broth, respectively (Table 1). To our knowledge, this is the first report that *R. stabekisii* and *A. tumefaciens* were able to degrade histamine.

3.2. Effect of temperature on the growth and histamine degradation activity of *B. polymyxa* D05-1

In this study, *B. polymyxa* D05-1 isolate exhibited the highest histamine degradation activity in histamine broth. Therefore, *B. polymyxa* D05-1 was evaluated for growth and histamine degradation activity under different conditions. The growth and histamine degradation activity of *B. polymyxa* incubated at various temperatures in histamine broth are shown in Fig. 1. As shown in Fig. 1A, *B. polymyxa* grew well in histamine broth during incubation at 30°C and 37°C. However, the highest bacterial count of 8.8 log CFU/mL was detected at 30°C after 24 hours (Fig. 1A). The bacterial counts of *B. polymyxa* incubated at 15°C and 25°C gradually increased until they reached approximately 7.9 log CFU/mL after 12 hours and 8.0 log CFU/mL after 24 hours, respectively. However, growth of *B. polymyxa* was retarded at 4°C and completely inhibited at 45°C. In agreement with our result, Zaman et al [20] reported that the histamine-degrading isolate, *S. carnosus*, isolated from fish sauce grew well at 35°C, but could not grow at 45°C.

As shown in Fig. 1B, the highest histamine dehydrogenase activity (1.15 U/mg) of *B. polymyxa* was observed at 30°C after 24 hours of incubation. The second highest enzyme activity (1.05 U/mg) of *B. polymyxa* was observed after 48 hours of incubation at 37°C followed by incubation at 25°C and then at 15°C. No enzyme activity was detected in histamine broth

incubated at 4°C and 45°C for 48 hours. The histamine-degrading capability (%) of *B. polymyxa* in histamine TSB broth reached 100% at 25°C, 30°C, and 37°C after 6 hours of incubation (Fig. 1C). At 15°C for 6 hours of incubation, 58% of histamine was degraded, whereas no histamine was degraded in histamine broth incubated at 4°C and 45°C for 48 hours. However, the highest histamine-degrading enzyme activity of the halophilic archaeon, *N. gari*, isolated from fish sauce was observed at 40–55°C [12]. The highest histamine-degrading percentage of *S. carnosus*, isolated from fish sauce was 23.5% at 40°C after 24 hours incubation [20]. The difference among temperatures for the highest histamine-degrading capability could be due to different species of microbial histamine-degrading bacteria. In general, most of the growth-associated enzymes are known to exhibit the optimal activity at the optimal temperature for growth [20].

3.3. *Effect of pH on the growth and histamine degradation activity of B. polymyxa D05-1*

The growth and histamine degradation activity of *B. polymyxa* incubated in various pH of histamine broth at 30°C for 24 hours are shown in Fig. 2. *B. polymyxa* exhibited tolerance to a broad range of pH values, and its optimal pH of growth was observed from pH 5 to pH 9 (Fig. 2A). A decrease in the bacterial count of approximately four log cycles (compared to that at the optimal pH) was observed in broth with pH 4 or pH 10. At pH 2 and pH 11, bacterial growth was completely inhibited. Similar to bacterial growth, the highest histamine dehydrogenase activity (1.16 U/mg) of *B. polymyxa* was observed in the pH range of 5–9 at 30°C for 24 hours (Fig. 2B). At pH 4 and pH 10, low enzyme activity (<0.05 U/mg) was observed in broth. No histamine-dehydrogenase activity was detected at pH < 3 and pH 11 in histamine broth. As shown at Fig. 2C, the highest histamine-degrading capability (100%) was observed at pH 7 in histamine broth incubated at 30°C for 24 hours. Either an acidic (pH 5–6) or alkaline (pH 8–9) condition slightly inhibited the histamine-degrading capability (72–82%) of *B. polymyxa*, whereas only 7.5% and 35% of histamine were degraded in broth at pH 4 and pH 10, respectively. These results are in agreement with a previous report of histamine degradation by *N. gari* isolated from fish sauce that exhibited optimal degradation activity at pH 6.5–7.5 [13]. In contrast, the pH optimum of histamine dehydrogenase activity from *B. polymyxa* in this study was lower than those from *Alcaligenes xylosoxidans* (pH 8.0) [21], *N. simplex* IFO 12069 (pH 8.5) [22], and *Rhizobium* sp. 4-9 (pH 9.0) [18].

3.4. *Effect of salt concentration on the growth and histamine degradation activity of B. polymyxa D05-1*

The growth and histamine degradation activity of *B. polymyxa* incubated in various salt concentrations of histamine broth at 30°C for 24 hours are shown in Fig. 3. *B. polymyxa* exhibited tolerance to a broad range of salt concentrations, and its optimal salt concentration of growth was observed at 0.5% (8.8 log CFU/mL; Fig. 3A). At 5% NaCl, bacterial count was significantly decreased to 7.3 log CFU/mL. However, as the NaCl content in the medium increased 10–20%, bacterial growth was significantly inhibited and retarded approximately 4.0 log CFU/mL. As shown in Fig. 3B, the highest histamine dehydrogenase activity (1.16 U/mg) of *B. polymyxa* was observed at 0.5% of NaCl, followed by 5.0% (1.13 U/mg). At 10% NaCl, low enzyme activity (0.05 U/mg) was observed in cultured broth. No histamine dehydrogenase activity was detected in histamine broth containing 15% or 20% NaCl. Similar to histamine dehydrogenase activity, the highest histamine-degrading capability (100%) was observed at 0.5% NaCl in histamine broth incubated at 30°C for 24 hours. A higher salt concentration (5%) slightly reduced the histamine-degrading capability (97%) of *B. polymyxa*, whereas only 8.5% of histamine was degraded in cultured broth containing 10% NaCl. No histamine was degraded by *B. polymyxa* in cultured broth at 15% and 20% NaCl. Thus, *B. polymyxa* D05-1 may be considered a halotolerant histamine-degrading bacterium based on the salt tolerance of bacterial growth, and levels of NaCl in excess of 10% inhibited their histamine-degrading activity in this study. However, Zaman et al [20] found that histamine degradation by *S. sarnosus* FS19 isolated from fish sauce was optimal in medium containing 9% NaCl. Sinsuwan et al [23] also demonstrated that histamine degradation by *Brevibacillus* sp. SK35 isolated from fish sauce was optimal in medium containing 10% salt. The optimal activity of the histamine-degrading enzymes of *N. gari* HDS3-1 was observed in the presence of 15–19% NaCl [12]. The difference between salt concentrations for optimal histamine-degrading activity could be due to the use of different species of microbial histamine-degrading bacteria.

Recently, Mah and Hwang [11] studied biogenic amine reduction in Myeolchi-jeot, a salted and fermented anchovy (*Engraulis japonicas*), by applying starter cultures during ripening. *S. xylosus* No 0538 degraded histamine and tyramine by 38% and 4%, respectively, and the total biogenic amine level was decreased by 16% [11]. After 120 days of fermentation in fish sauce, the histamine concentrations were reduced by 27.7% and 15.7% in samples inoculated with *S. carnosus* FS19 and *B. amyloliquefaciens* FS05, respectively, compared to control samples [24]. In Taiwan, some salted fish products had histamine contents greater than the 5.0 mg/100g allowable limit suggested by the US Food and Drug Administration [2]. Therefore, the histamine-degrading isolate, *B. polymyxa* D05-1, isolated from Taiwanese salted fish product in this study, might be used as a starter culture in inhibiting histamine accumulation during salted fish product fermentation.

4. Conclusion

Eight histamine-degrading bacteria isolated from salted fish samples exhibited the ability to degrade histamine in histamine TSB broth. Among them, *B. polymyxa* D05-1 was able to degrade histamine up to 100% in histamine TSB broth. Moreover, *B. polymyxa* D05-1 can be considered a halotolerant histamine-degrading bacterium and produces histamine dehydrogenase to degrade histamine. The optimal growth, histamine dehydrogenase activity, and histamine degrading capability of *B. polymyxa* were observed after incubation in a medium of pH 7.0 with 0.5% NaCl at 30°C for 24 hours.

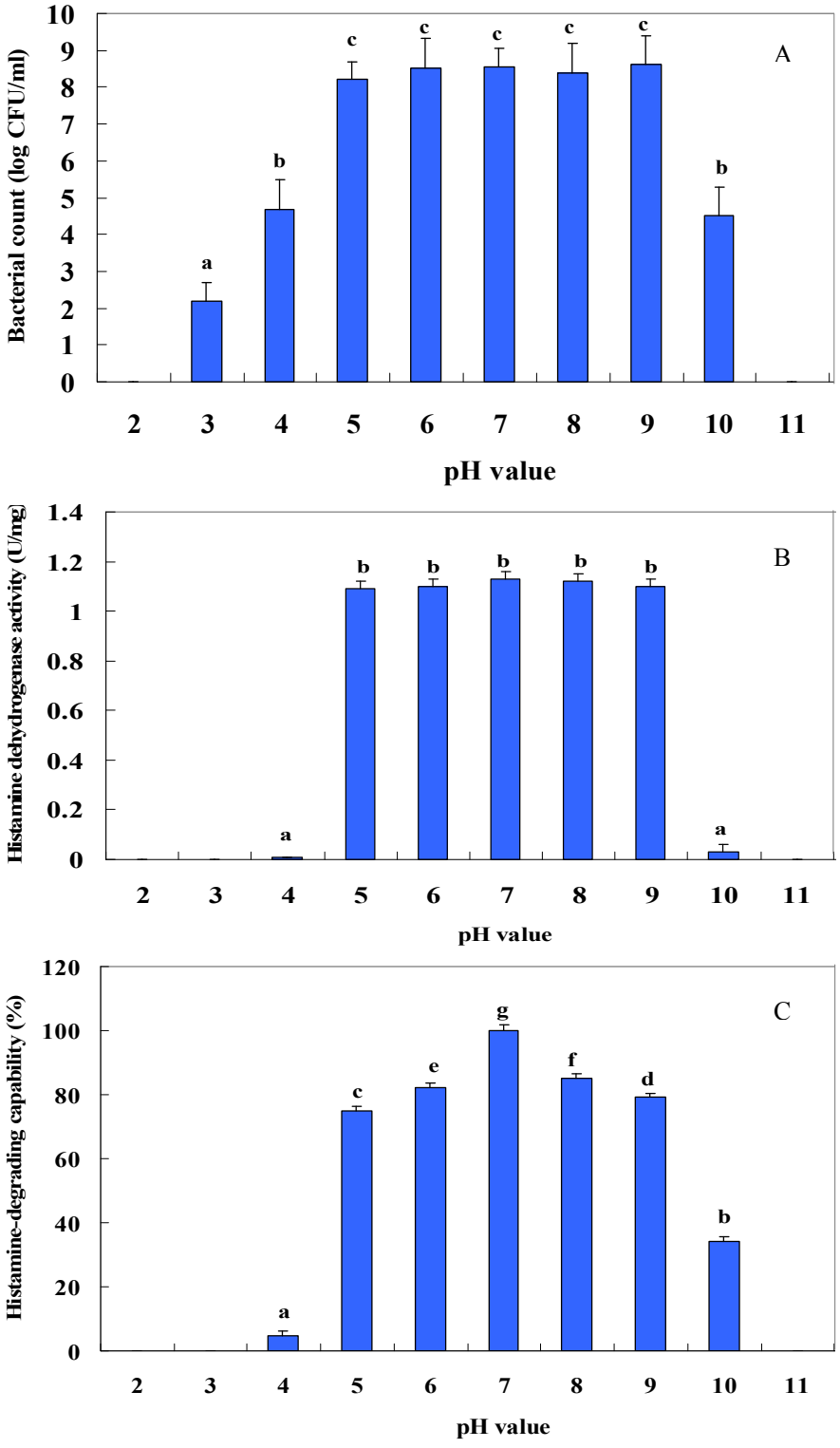

Fig. 2 – Effect of pH on (A) growth, (B) histamine dehydrogenase activity, and (C) histamine-degrading capability of *Bacillus polymyxa* D05-1 incubated in histamine broth at 30°C for 24 hours. Error bars represent standard deviation of three replicates. Bars labeled with different letters are significantly different ($p < 0.05$).

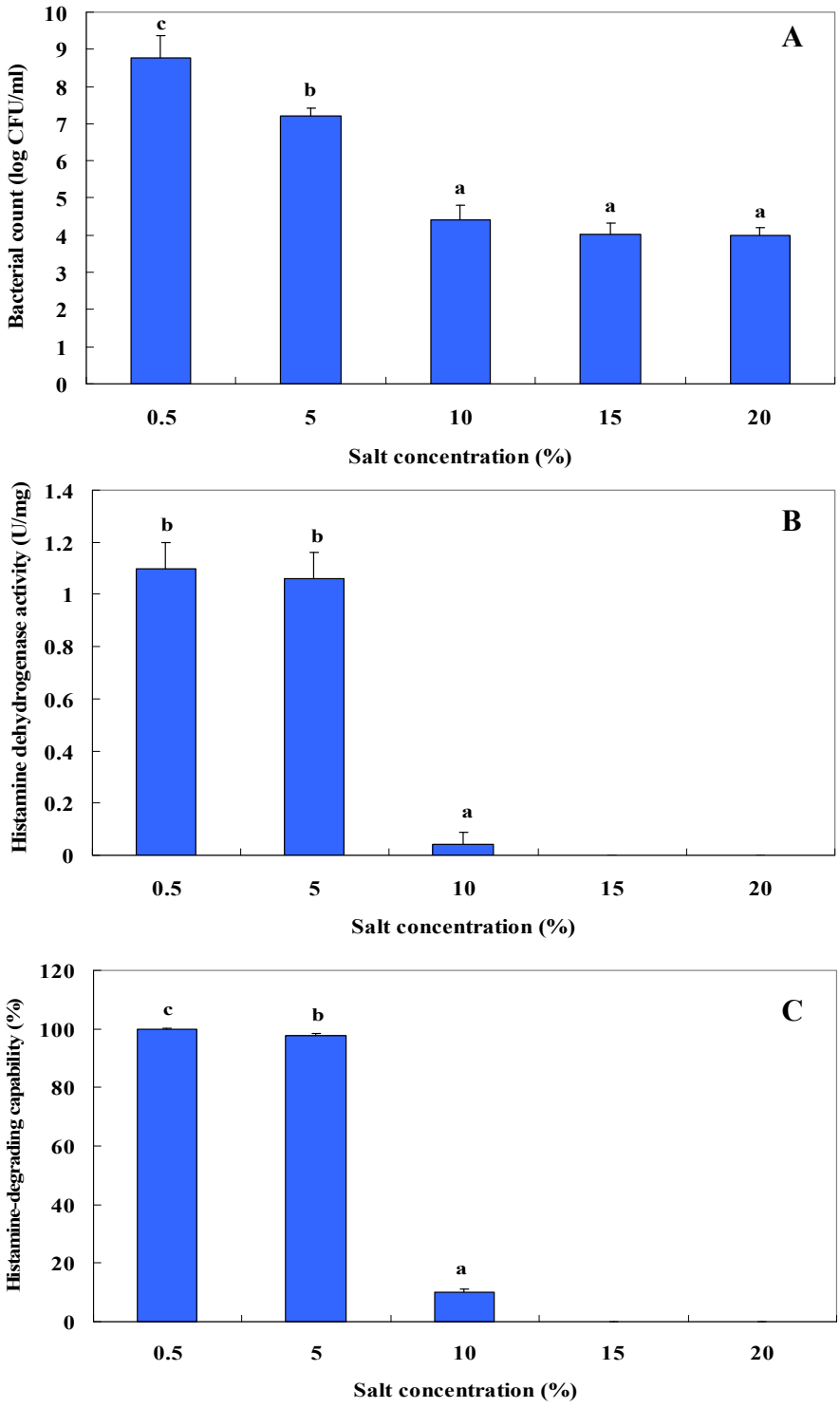

Fig. 3 – Effect of salt concentration on (A) growth, (B) histamine dehydrogenase activity, and (C) histamine-degrading capability of *Bacillus polymyxa* D05-1 incubated in histamine broth at 30°C for 24 hours. Error bars represent standard deviation of three replicates. Bars labeled with different letters are significantly different ($p < 0.05$).

Conflicts of interest

All authors declare no conflicts of interest.

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