Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Mitigating biogenic amine accumulation in fish: Potential of *Bacillus polymyxa* as a biocontrol strategy

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ARTICLE INFO

Keywords

Biocontrol

Biogenic Amines

Fish products

Histamine

Received 15/12/2024
Accepted 04/02/2025
Available On-Line 01/04/2025

ABSTRACT

Nitrogen compounds known as biogenic amines, particularly histamine, putrescine, and tyramine, are formed in fish and fish products through the decarboxylation of free amino acids. Their accumulation is affected by microbial proliferation, unsanitary conditions, and improper temperature management during storage. While histamine is linked to scombroid food poisoning, putrescine and cadaverine may enhance its toxic effects. This research examined the potential of *Bacillus polymyxa* as a biocontrol method to reduce biogenic amines in fish fillets. The study utilized fresh fish fillets divided into six groups: three control groups containing standard solutions of histamine, putrescine, and tyramine (40 mg/100g each), and three treatment groups inoculated with *B. polymyxa* (5×107 bacteria) along with each biogenic amine standard solution. Samples were incubated for 36 h, with biogenic amine levels measured at 0, 12, 24, and 36 h intervals. The application of *B. polymyxa* resulted in a substantial decrease in histamine, putrescine, and tyramine levels in the treated fish fillets, with reduction rates of 75.2%, 79.5%, and 65.3%, respectively, after 36 hours, respectively. These results indicate that *B. polymyxa* could an effective biocontrol agent for minimizing biogenic amines in fish products, thus improving their safety and quality. Additional studies are necessary to evaluate its efficacy across various fish species and processing conditions.

1. INTRODUCTION

A balanced diet, including fish and fish products as vital components, offers diverse nutrients essential for human well-being. These foods are abundant in premium protein, omega-3 fatty acids (specifically EPA and DHA), vitamins (notably B12 and D), and minerals like selenium, iodine, and calcium (Mæhre et al., 2016; Jeyasekaran and Shakila, 2022). The nutritional benefits of fish go beyond protein content, with fatty fish serving as a particularly valuable source of omega-3 fatty acids and vitamin D (Jeyasekaran and Shakila, 2022).

While fish consumption is generally linked to numerous health advantages, such as decreased risk of heart disease, enhanced neurological development in fetuses, and protection against various ailments (Jeyasekaran and Shakila, 2022), it is important to consider potential risks as well.

Biogenic amines, which are nitrogen-containing compounds formed through the decarboxylation of free amino acids, primarily result from bacterial activity (Madejska et al., 2017). In fish and fish products, the most significant biogenic amines are histamine, cadaverine, and putrescine, which play key roles in ensuring fish safety and quality (Bulushi et al., 2009). The build-up of these amines is largely attributed to the growth of bacteria with amino acid decarboxylase activity, often worsened by poor hygiene and improper temperature management during storage (Abuhlega and Ali, 2022).

Notably, while histamine is commonly associated with scombroid food poisoning, it may not be sufficient on its own to cause toxicity. Research suggests that putrescine and cadaverine may enhance histamine toxicity (Bulushi et al. 2009). Furthermore, the interplay between biogenic amines, sensory evaluation, and trimethylamine during spoilage is influenced by the bacterial composition and free amino acid content (Bulushi et al., 2009). In certain fish species, such as the Mediterranean hake, cadaverine has been identified as a specific spoilage biogenic amine during refrigerated storage (Baixas-Nogueras et al., 2005). Consequently, this research aimed to examine the impact of *Bacillus polymyxa*on various biogenic amines in fish fillets as a biocontrol strategy.

2. MATERIAL AND METHODS

All procedures in this study was approved by Scientific Research Ethics Committee, Faculty of Medicine, Benha University with Ethical Approval Number (BUFVTM16-09-24).

2.1. Bacterial Strain Preparation

To prepare an overnight culture, the *Bacillus polymyxa* strain(Food Analysis Center, Faculty of Veterinary Medicine, Benha Univerity) as grown in Brain Heart Infusion (BHI) broth (Fluka, Sigma-Aldrich Chemie GmbH) for 24 hours at 37 °C. The cultivated bacterial suspension was then serially diluted in sterile peptone water (0.1%, w/v) (Merck, Darmstadt, Germany) using 1 mL of the suspension. The viable count of the *Bacillus polymyxa* strain was subsequently determined using the plate count method. (ISO, 2013) Approximately 5×10^7 bacteria from the culture broth were centrifuged (500 rpm, 15 minutes at 5 ºC), and the resulting bacterial pellets were washed twice with deionized water (Halttunen et al., 2007).

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2.2. Binding assay (Halttunen et al., 2008)

Bacterial suspensions (5×10^7cfu/g) were used to inoculate 2 kg of raw fish from *Tilapia niloticus*. The specimens were divided into six distinct groups. For the first three groups, each was treated separately with a standardized solution containing 40 mg/100 g of histamine, putrescine, and tyramine(Milex, Massachusetts, USA). The fourth group received a mixture of 5×10^7 bacteria and 40 mg/100 g histamine standard solution. The fifth group was treated with 5×10^7 bacteria and 40 mg/100 g putrescine, while the sixth group received 5×10^7 bacteria and 40 mg/100 g tyramine. The bacterial pellets and biogenic amine solutions were briefly vortexed (Stuart, Staffordshire, U.K) for 5 seconds and then incubated for 24 hours on a Finemixer SH2000 orbital shaker (Finepcr, Seoul, Korea) with gentle agitation. Control samples consisted of fish contaminated with histamine, putrescine, and tyramine, as well as samples without *Bacillus polymyxa*.

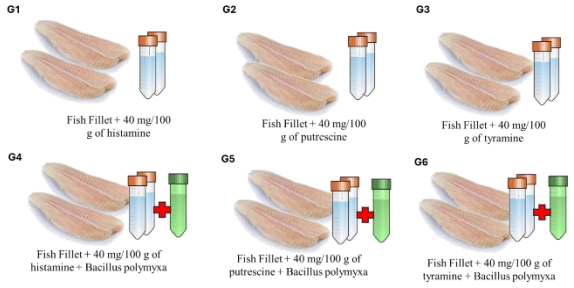

Figure 1 Fish Fillet Samples preparation

The samples were acidified using ultrapure HNO3 and analyzed at 0, 12, 24, and 36 hours to determine histamine, putrescine, and tyramine concentrations. The percentage reduction of biogenic amines in the treated fish fillets was calculated and documented.

2.3. Statistical analysis

The data gathered was assessed through ANOVA with Duncan using SPSS version 16.0 as outlined by Feldman et al (2003).

3. RESULTS

Bacillus polymyxa was evaluated as a biological agent to reduce biogenic amine concentrations, including histamine, putrescine, and tyramine, in fish fillets through experimental inoculation. The data in Table (1) highlights the effect of *Bacillus polymyxa* culture (5×10^7) on the histamine content in fish fillets experimentally inoculated with 40 mg/100 g. Over a storage period of 36 hours, the histamine levels in the treated group decreased as compared to the untreated control group. At zero time, no reduction was observed as the histamine concentration remained at 40 mg/kg for both groups. However, by 12 hours, the treated group exhibited a 36.8% reduction in histamine levels (to 25.3 mg/kg), which progressively improved, reaching a reduction of 58.3% at 24 hours (16.7 mg/kg) and a substantial 75.2% reduction at 36 hours (9.9 mg/kg).

Table 1 Impact of *B. polymyxa* culture ($5x10^7$) on the reduction of histamine experimentally introduced into fish fillets (40 mg/100 g).

Storage time	Control (mg/Kg)	*B. polymyxa* treated group (mg/Kg)	Reduction %
Zero time	40	40	-------
12 hours	40	25.3	36.8
24 hours	40	16.7	58.3
36 hours	40	9.9	75.2

Table (2) showed the impact of *B. polymyxa* (5×10^7)cfu/gm on another trial involving putrescine in fish fillets, similarly, inoculated at 40 mg/100 g. The trend mirrors the findings of Table 1, with no immediate reduction at zero time but significant decreases observed over time. After 12 hours, putrescine levels in the treated group dropped to 21.6 mg/kg, corresponding to a 46% reduction, while at 24 hours, the level decreased further to 14.5 mg/kg (63.8% reduction). By 36 hours, the treated group exhibited the highest reduction of 79.5% (8.2 mg/kg). The enhanced reduction percentages compared to Table (1) reaffirming potent histamine-degrading properties of *B. polymyxa.*

Table 2 Impact of *B. polymyxa* culture ($5x10^7$) on the reduction of putrescine experimentally introduced into fish fillets (40 mg/100 g)(n=10)

Storage time	Control (mg/Kg)	*B. polymyxa* Treated group (mg/Kg)	Reduction %
Zero time	40	40	-------
12 hours	40	21.6	46
24 hours	40	14.5	63.8
36 hours	40	8.2	79.5

The effect of *B. polymyxa* (5×10^7) on tyramine levels in fish fillets, experimentally inoculated at 40 mg/100 g, is presented in table (3). Actually, tyramine degradation by *B. polymyxa* occurred with variable rate but was still substantial over the 36-hour storage period. At zero time, no reduction was noted. By 12 hours, the treated group exhibited a 29.8% reduction in tyramine levels (28.1 mg/kg). At 24 hours, the reduction increased to 48.5% (20.6 mg/kg) and further to 65.3% (13.9 mg/kg) by 36 hours. Accordingly, the obtained date indicated the broad-spectrum effective action of *B. polymyxa* in reducing biogenic amines, which could enhance the safety and quality of stored fish products.

Table (3) Impact of *B. polymyxa* culture ($5x10^7$) on the reduction of tyramine experimentally introduced into fish fillets (40 mg/100 g)(n=10).

Storage time	Control (mg/Kg)	*B. polymyxa* Treated group (mg/Kg)	Reduction %
Zero time	40	40	-------
12 hours	40	28.1	29.8
24 hours	40	20.6	48.5
36 hours	40	13.9	65.3

4. DISCUSSION

In this study the impact of *Bacillus polymyxa* on various biogenic amines in fish fillets as a biocontrol strategy were examined. Biogenic amines serve as crucial indicators of fish quality and freshness. Their concentrations differ among fish species and are affected by various factors, including storage conditions, processing techniques, and microbial activity (Zhai et al., 2011; Fusek et al., 2020). While these compounds can present health hazards, such as histamine poisoning and potential nitrosamine formation (Bulushi et al., 2009), they also offer valuable insights into the hygienic status of diverse marine and freshwater species (Prester, 2011). Ensuring food safety and maintaining product quality necessitates the monitoring and regulation of biogenic amine levels in fish and fish-derived products.

Bacillus polymyxa, extracted from salted fish products, has demonstrated promising results in managing biogenic amine accumulation during fish storage (Kung et al., 2015). Research has shown its efficacy in breaking down histamine and slowing the increase of total volatile basic nitrogen in salted fish products (Kung et al., 2015; Lee et al., 2015). When employed as a starter culture in salted fish fermentation at 35 °C for 120 days, *B. polymyxa* significantly lowered the levels of total volatile basic nitrogen and overall biogenic amine content, including histamine, putrescine, cadaverine, and tyramine, in comparison to control samples (Lee et al., 2015). The

efficacy of *Bacillus polymyxa* as a biological agent to mitigate biogenic amine accumulation in fish fillets was evaluated, with particular attention to histamine and putrescine degradation over a controlled storage period. The results presented in Table 1 demonstrate the significant histamine-degrading potential of *B. polymyxa* (5×10^7 CFU/ml). Initially, histamine levels in both treated and control groups were 40 mg/kg, confirming the baseline concentration and inoculation consistency. However, after 12 hours of storage, histamine levels in the treated samples were reduced by 36.8% to 25.3 mg/kg. This trend continued, with reductions of 58.3% and 75.2% recorded at 24 hours (16.7 mg/kg) and 36 hours (9.9 mg/kg), respectively, underscoring the sustained efficacy of *B. polymyxa* in histamine degradation under experimental conditions. Similar findings are reported in prior studies, where biocontrol agents such as Bacillus species have been shown to metabolize histamine effectively, likely through enzymatic pathways involving histidine decarboxylase inhibitors or oxidative deamination processes (Kim et al., 2019; Eom et al., 2020).

Furthermore, as illustrated in Table 2, B. polymyxa demonstrated comparable potency in reducing putrescine concentrations, initially inoculated at 40 mg/kg. Over the 36-hour storage period, putrescine levels in treated samples exhibited reductions of 46%, 63.8%, and 79.5% at 12 hours (21.6 mg/kg), 24 hours (14.5 mg/kg), and 36 hours (8.2 mg/kg), respectively. The slightly higher reduction percentages observed for putrescine compared to histamine may indicate differential substrate affinity or enzymatic activity by *B. polymyxa* under the prevailing experimental conditions. These findings align with existing literature, which highlights the role of specific Bacillus strains in degrading putrescine and other polyamines in protein-rich matrices, such as fish fillets, by modulating environmental pH and utilizing amine oxidases (Yew et al., 2014; Chang et al., 2018). Collectively, the data underscores the potential application of B. polymyxa as a biocontrol agent to enhance the safety and quality of fish products by significantly reducing biogenic amine levels during storage.

The majority of studies have concentrated on the ability of these bacteria to degrade histamine and other biogenic amines (Lee et al. 2015). This suggests that the putrescine-degrading activity of *B. polymyxa* may be part of its broader capacity to reduce various biogenic amines. The current study demonstrates that *B. polymyxa* shows promise as an effective biocontrol agent for reducing putrescine and other biogenic amines in fish products (Kung et al., 2015).

5. CONCLUSIONS

The current study demonstrated that *B. polymyxa* shows promise as an effective biocontrol for reducing biogenic amines in fish products, thus improving their quality. Nevertheless, additional studies are necessary to evaluate the efficacy of *B. polymyxa* across various fish species and processing conditions, as the formation of biogenic amines can differ substantially among fish products and storage environments.

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