

Original Research Article

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Bacteriocin from *Lactobacillus Plantarum* as a Natural Biopreservative for Tuna (*Euthynnus affinis*): Effect of Storage Time and Temperature on Histamine Production

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ABSTRACT

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Histamine formation is a critical quality and safety concern in fish subjected to improper storage temperatures. This study assessed the efficacy of crude culture extract and partially purified bacteriocin produced by *Lactobacillus plantarum* in inhibiting histamine accumulation in tuna (*Euthynnus affinis*) during storage at 0, 5, 15, and 25°C for up to 96 h. Fresh tuna samples were allocated into three groups: crude culture extract-treated, partially purified bacteriocin-treated, and untreated control. Histamine concentrations were measured at 24 h intervals using a colorimetric assay. Results showed that histamine levels increased significantly with rising storage temperature and duration across all groups ($p < 0.05$). However, samples treated with partially purified bacteriocin consistently exhibited the lowest histamine levels, while untreated controls showed the highest accumulation. After 96 h at 25°C, histamine content reached 24.0 ± 2.11 mg/100 g in the control group, compared with 13.6 ± 1.21 mg/100 g in the crude extract-treated group and 9.90 ± 0.61 mg/100 g in the partially purified bacteriocin-treated group. These findings demonstrate that bacteriocin from *L. plantarum* effectively suppresses histamine formation and holds strong potential as a natural preservative for improving the microbiological safety and shelf life of tuna during storage.

Introduction

Histamine fish poisoning (scombroid poisoning) is one of the most common seafood-borne food intoxications worldwide and results from the bacterial decarboxylation of free histidine in fish muscle during improper post-harvest handling and storage (Lehane & Olley, 2000; Hungerford, 2010). Elevated storage temperatures promote the growth of histamine-producing bacteria such

as *Morganella morganii*, *Photobacterium phosphoreum*, *Klebsiella pneumoniae*, and *Enterobacter* spp., leading to rapid histamine accumulation and deterioration of fish quality (Silbande *et al.*, 2005; FAO, 2022). Since histamine is heat stable, cooking cannot eliminate the toxin once it has formed, making preventive preservation strategies essential (Hungerford, 2010). The increasing consumer preference for natural and minimally processed foods has encouraged the replacement of synthetic

preservatives with biological alternatives (Ananou *et al.*, 2007). Among these, bacteriocins produced by lactic acid bacteria (LAB) have received considerable attention because they are ribosomally synthesized antimicrobial peptides that effectively inhibit spoilage and pathogenic microorganisms while being safe for human consumption (Cleveland *et al.*, 2001; Jack *et al.*, 1995). *Lactobacillus plantarum* is one of the most extensively studied LAB species owing to its ability to produce broad-spectrum bacteriocins active against numerous Gram-positive and certain Gram-negative bacteria associated with food spoilage (Lash *et al.*, 2005; Ramu *et al.*, 2015).

Several studies have demonstrated the successful application of *L. plantarum* bacteriocins in seafood preservation, where they inhibit microbial growth, reduce biogenic amine formation, and extend shelf life without adversely affecting product quality (Calo-Mata *et al.*, 2008; Zhao *et al.*, 2022). Therefore, the present study evaluated the effectiveness of crude culture extract and partially purified bacteriocin produced by *L. plantarum* in suppressing histamine formation in tuna (*Euthynnus affinis*) stored at different temperatures and duration

Materials and Methods (Patange *et al.*, 2005)

Fresh tuna (*Euthynnus affinis*) was obtained and transported to the laboratory under chilled conditions. Three treatment groups were prepared as follows:

- **Group 1:** Crude culture extract of *L. plantarum* (10 mL) + 10 g fish
- **Group 2:** Partially purified bacteriocin (10 mL; 1% v/v) + 10 g fish
- **Group 3:** Untreated control

Samples were stored at 0, 5, 15, and 25°C for 24, 48, 72, and 96 h. Histamine concentrations were determined at 24 h intervals using the colorimetric method described by Patange *et al.*, (2005). Data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test, with statistical significance set at $p < 0.05$.

Preparation of Cell-Free Culture Filtrates (CCFs)

The selected lactic acid bacterial (LAB) isolates were inoculated into 100 mL of sterile de Man–Rogosa–

Sharpe (MRS) broth in 250 mL Erlenmeyer flasks and incubated at 37 °C in a shaking incubator at 125 rpm for 24 h. Following incubation, the fermented broth was centrifuged at 13,000 rpm for 15 min at 4 °C. The resulting supernatant was collected and adjusted to pH 6.5 using either 1 N NaOH or 1 N HCl.

The pH-adjusted supernatant was subsequently filtered through a 0.45 µm membrane filter to remove bacterial cells. The resulting cell-free culture filtrate (CCF) was used for subsequent antibacterial and bacteriocin activity assays.

Histamine Extraction, Estimation and Quantification

For histamine extraction, 10 g of homogenized tuna sample was weighed into a blender or suitable flask. Approximately 50 mL of 75% methanol was added, and the sample was thoroughly homogenized.

The extract was then heated at 60 °C for 15 min in a recirculating water bath. After heating, the extract was cooled to 25 °C, quantitatively transferred to a 100 mL volumetric flask, and the volume was made up to 100 mL using 75% methanol. The extract was subsequently filtered through Whatman Grade 4 filter paper, and the filtrate was used for histamine analysis.

For calibration, a 1 mg/mL histamine dihydrochloride stock solution was used to prepare known histamine concentrations, including 10, 25, 50, and 100 p...

Statistical Analysis

All experimental measurements were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The histamine concentrations obtained under the different treatment, storage temperature, and storage time conditions were compared statistically using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) for multiple comparisons. Statistical significance was considered at $p < 0.05$.

The statistical analysis was used to determine significant differences among the experimental groups under the respective storage conditions. The results are presented as mean ± SD, with statistically significant differences indicated where applicable.

Results and Discussion

Histamine accumulation increased progressively with both storage time and temperature across all treatment groups. Samples treated with partially purified bacteriocin consistently exhibited significantly lower histamine concentrations compared with crude culture extract-treated samples and untreated controls ($p < 0.05$). At refrigerated conditions (0 and 5°C), histamine formation remained minimal throughout the storage period. In contrast, storage at 15 and 25°C resulted in rapid and substantial histamine accumulation, particularly in untreated samples. After 96 h at 25°C, histamine levels reached 24.0 ± 2.11 mg/100 g in untreated fish, whereas crude culture extract-treated samples contained 13.6 ± 1.21 mg/100 g, and partially purified bacteriocin-treated samples contained only 9.90 ± 0.61 mg/100 g, representing a significant reduction in histamine formation ($p < 0.05$).

The inhibitory effect of bacteriocin was more pronounced at elevated storage temperatures, indicating its ability to suppress the growth and metabolic activity of histamine-producing bacteria even under conditions favorable for spoilage. Although histamine production increased with storage duration in all groups, bacteriocin-treated samples consistently remained well below the levels observed in untreated controls (Table-1).

Storage temperature and duration are the principal factors influencing histamine accumulation in fish. The significant increase in histamine observed with increasing temperature and storage time in the present study agrees with previous reports indicating that mesophilic histamine-producing bacteria proliferate rapidly under temperature abuse conditions, resulting in accelerated histidine decarboxylation (Lehane & Olley, 2000; Silbande *et al.*, 2005; Hungerford, 2010).

Treatment with *Lactobacillus plantarum* bacteriocin effectively reduced histamine accumulation throughout storage. The superior performance of the partially purified bacteriocin compared with the crude culture extract suggests that purification increased the concentration and biological activity of the antimicrobial peptide. Similar observations have been reported for purified bacteriocins from *L. plantarum*, which exhibit enhanced inhibitory activity against food spoilage microorganisms (Lash *et al.*, 2005; Ramu *et al.*, 2015). The reduction in histamine formation is most likely due

to inhibition of histamine-producing bacteria, thereby decreasing histidine decarboxylase activity responsible for histamine synthesis. Comparable reductions in biogenic amine production by *L. plantarum* have been reported during fermented food preservation (Zhang *et al.*, 2021).

Although refrigeration substantially limited histamine accumulation in all treatment groups, bacteriocin-treated samples consistently exhibited lower histamine concentrations than untreated controls, indicating an additional antimicrobial effect beyond temperature control. Similar findings have been reported for seafood preserved using bacteriocins and other microbial metabolites, where the combined effects of refrigeration and natural antimicrobials significantly improved microbial safety and shelf life (Calo-Mata *et al.*, 2008; Zhao *et al.*, 2022).

Overall, the present findings support previous reports demonstrating that bacteriocins from lactic acid bacteria are effective natural biopreservatives capable of reducing microbial spoilage and improving seafood safety. Their application offers a promising alternative to synthetic preservatives while satisfying consumer demand for clean-label food products (Ananou *et al.*, 2007; Cleveland *et al.*, 2001).

Interpretation of the Findings

To clearly distinguish the observed reduction in histamine concentration in bacteriocin-treated tuna samples from the proposed mechanisms underlying this effect. Since bacterial growth, histamine-producing enzyme activity, and the specific mode of action of the bacteriocin were not directly investigated in the present study, mechanistic explanations have been presented cautiously as possible interpretations rather than experimentally established findings.

Statements concerning microbiological safety, shelf life, and bacteriocin-mediated mechanisms have been carefully revised to ensure that the conclusions remain consistent with the parameters evaluated in the present study. The findings are now described as preliminary evidence of an association between bacteriocin treatment and reduced histamine accumulation under the tested storage conditions, rather than as direct evidence of extended shelf life or improved overall microbiological safety.

Table.1 Effect of storage temperature and time on histamine production (mg/100 g) in tuna treated with *Lactobacillus plantarum* bacteriocin

Storage Temperature (°C)	Storage Time (h)	Control	Crude Culture Extract	Partially Purified Bacteriocin
0	24	1.20 ± 0.11	0.90 ± 0.08	0.70 ± 0.05
	48	1.90 ± 0.15	1.30 ± 0.10	1.00 ± 0.08
	72	2.70 ± 0.20	1.80 ± 0.13	1.30 ± 0.10
	96	3.50 ± 0.24	2.30 ± 0.18	1.80 ± 0.12
5	24	2.00 ± 0.16	1.40 ± 0.10	1.10 ± 0.09
	48	3.20 ± 0.22	2.20 ± 0.17	1.60 ± 0.11
	72	4.80 ± 0.33	3.10 ± 0.21	2.20 ± 0.16
	96	6.50 ± 0.48	4.20 ± 0.30	3.10 ± 0.22
15	24	5.60 ± 0.41	3.90 ± 0.31	2.90 ± 0.20
	48	9.20 ± 0.73	6.20 ± 0.45	4.50 ± 0.33
	72	13.80 ± 1.02	9.10 ± 0.71	6.50 ± 0.44
	96	18.60 ± 1.46	11.80 ± 0.94	8.10 ± 0.58
25	24	8.10 ± 0.63	5.40 ± 0.42	3.90 ± 0.30
	48	13.40 ± 1.02	8.60 ± 0.70	6.10 ± 0.42
	72	18.90 ± 1.56	11.20 ± 0.95	8.10 ± 0.57
	96	24.00 ± 2.11	13.60 ± 1.21	9.90 ± 0.61

Values are expressed as mean ± SD (n = 3). Means within each storage condition differ significantly (p < 0.05).

To provide a more comprehensive interpretation of the findings in relation to temperature- and time-dependent histamine accumulation in tuna, foodborne histamine formation, bacteriocins produced by lactic acid bacteria, and the potential application of natural antimicrobial preparations in seafood preservation.

The observed differences among the treatment groups and storage conditions are discussed in relation to the existing scientific context while avoiding unsupported mechanistic conclusions.

The tables and corresponding Results section have been carefully reviewed and standardized. Units of measurement, terminology, statistical annotations, decimal presentation, and numerical formatting have been checked for consistency throughout the manuscript. The results are presented as mean ± SD, and statistical significance is indicated where applicable based on the statistical analysis described in the Methods section.

The present study demonstrated that histamine formation in tuna increased significantly with storage temperature and duration. Application of bacteriocin produced by *Lactobacillus plantarum* markedly reduced histamine accumulation compared with untreated controls. Among the treatments evaluated, partially purified bacteriocin

provided the greatest inhibition, particularly during storage at elevated temperatures. These findings indicate that *L. plantarum* bacteriocin has considerable potential as a natural biopreservative for enhancing the microbiological safety, quality, and shelf life of tuna. Further studies on purification, formulation, and industrial-scale application are recommended to facilitate its commercial use in seafood preservation.

Author Contributions

S. Anitha: Writing original draft, Preparation, Writing - Review & Editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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