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Original Research

Efficacy of Preserving Sea Foods Using Marine *Lactobacillus*

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Abstract

Scombroid food poisoning is a food borne illness that results from eating spoiled (decayed) fish. Histamine is the causative agent of scombroid poisoning, a food borne chemical intoxication. Histamine is one of the main biogenic amines and it is heterocyclic and biologically active primary amine, formed post-mortem in the muscles of scombroid and non-scombroid fish. The present study was carried out to isolate histamine-producing bacteria from a local fish and to test antibacterial activity of mangrove isolates of *Lactobacillus* species against the histamine producing bacteria. Fresh tuna fish (*Euthinus affinis*) obtained from Parangipettai coast and they were divided in to three groups. One group of the fish samples were stored directly and another two group of the fish samples were dipped in cold distilled water containing bacteriocin of *Lactobacillus* sp. and partially purified bacteriocin (10 ml, 1% v/v) respectively. The fish samples, after treatment were stored at 5, 15, 20 and 25 ^{0}C for the period of 24, 48, 72 and 96 h, respectively. Histamine quantification was performed at an interval of 24 h for four days. According to the results obtained it was proved that mangrove isolates of *Lactobacillus* species having high antimicrobial activity against histamine-producing bacteria which is responsible for spoilage of sea foods.

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INTRODUCTION

Histamine fish poisoning (HFP) is a food borne chemical intoxication caused by eating spoiled or bacterially contaminated fish. The fish have become toxic due to the bacterial contamination, but they may still have a normal appearance and odour (Sapin-Jaloustre and Sapin-Jaloustre, 1957). Organic acids, including sorbic acid (*trans-trans*-2,4-hexadienoic acid), benzoic acid and acetic acid, are the most commonly used chemical preservatives of food and are antimicrobial agents (Piper *et al.,* 1998). Spoilage incidences occur due to the ability of the contaminating organisms to overcome the modern-day preservation technologies. Some food-borne pathogenic bacteria can survive in lightly preserved food, stored at refrigeration temperatures, and they may pose a health risk to consumers (Heintz, 1998). Some other chemical preservatives like sodium chloride and nitrite (Roberts *et al.,* 1991) produce nitrosamine, at refrigeration temperatures, which is highly toxic. This leads to the search for alternative preservative agents and the consumer-led demand for more natural food products has also provided an increased interest in food-grade preservatives of biological origin.

In this respect, special interest has been focused on bacteriocins produced by lactic acid bacteria (LAB). Because bacteriocins are produced by naturally occurring food borne organisms and inhibit many bacterial pathogens they might be useful as antibacterial agents. Bacteriocin can be used to inhibit food borne pathogens by formulation of viable cells of a bacteriocinogenic strains into the food, by addition of purified bacteriocin (Daeschel, 1989). Applications are based on the bacterial cells that are able to grow and produce bacteriocin at both refrigeration and abused temperatures. Different bacteria vary significantly in quantity of histamine production at different times and temperatures. Klausen and Huss (1987) studied growth and histamine production with *Morgonella morgonii* in histidine-containing broth and in mackerel. Following storage at temperatures (10-25 °C), a large amount of histamine was produced, whereas at low temperatures (0-5 °C) no growth took place. Behling and Taylor (1982), pointed out that fish exposed to 20ºC for a short period of 1 day yielded high level of histamine following subsequent storage at refrigeration temperatures. Smith *et al.* (1983) also found a more rapid

production of histamine when mackerels were iced after 24 h at ambient temperature. These workers studied single temperature abuse for a minimum of 24 h. However, what is happening in different time duration and temperature of exposure is not clear. Such information is very much required for the practical utility of the data in the processing industry. Therefore, the present study was undertaken to understand the relationship between the time and temperature and corresponding microbial synthesis of histamine in commonly available edible fish, tuna under laboratory conditions treated with crude and partially purified bacteriocin of *Lactobacillus plantarum* as preservative agents.

MATERIALS AND METHODS

Test Organism and Material

The potential marine *lactobacillus*, *L. plantarum* and the partially purified bacteriocin were used in this study.

Fish Sample

Fresh tuna fish (*Euthinus affinis*) obtained from Parangipettai coast. The fish sample was placed into plastic bag on ice and brought to the laboratory. A representative 10 g of fish meat sample was taken aseptically and used for following preservation studies.

Preservation Studies Using Bacteriocin: Effect of Time and Temperature on Histamine Production

The effect of bacteriocin on preservation of the tuna fish, maintained under different time and temperature on the microbial synthesis of histamine was studied. The fish samples, after treatment were stored at 5, 15, 20 and 25 ^{0}C for the period of 24, 48, 72 and 96 h respectively. Histamine quantification was performed at on interval of 24 h for four days.

Experimental Design

The experimental procedures were followed as described by Arokiyamary and Sivakumar (2012).

Group 1: Crude culture extract of *L . plantarum* (10 ml) + 10 g fish sample

Group 2: Partially purified bacteriocin (10 ml, 1% v/v) + 10 g fish sample

Group 3: Control 10 g fish sample alone(no preservatives)

Analysis of Histamine from Spoiling Fish Samples

Histamine was estimated by new colorimetric assay (Patange *et al.,* 2005). The reagent, p-phenyldiazonium sulfonate was prepared according to Koessler and Hanke (1919) with minor modifications. The sample was homogenized with 20 ml of 0.85% NaCl solution (saline) for 2 min using a high-speed blender and centrifuged at 12000 X g for 10 min at 4°C. The supernatant was made up to 25 ml with saline. The muscle extract was used immediately for further analysis. In a clean tube, 5 ml of 1.1% sodium carbonate solution was taken and 2 ml of the chilled reagent was added slowly and mixed. It was then added to the tube containing 1 ml solution of the residue collected in the extraction process. The absorbance of the colour produced was measured with the help of UV/Vis spectrophotometer immediately after 5 min at 496 nm using distilled water as a blank. One ml aliquots of standard histamine solution containing 0–100 µg/ml in distilled water was reacted in a similar manner to obtain the reference colour scale and standard graph of absorbance against histamine concentration. The concentration of histamine in the unknown sample was detected with help of standard graph.

Calculation

Histamine in 100 g fish sample = the amount of histamine (µg/ml) ×^{a}25×^{b}20

^{a} 5 g fish sample extracted in 25 ml buffer

^{b} 5 g fish sample × 20 for 100 g conversion

Statistical Analysis

All data were expressed as Mean±Standard Deviation (SD) of the number of experiments (n=3). Statistical analysis was done by using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The fish samples preserved with partially purified bacteriocin (Group 2) had the lowest histamine content in all the tested temperature ranges when compared to the fish samples treated with crude culture filtrate of *Lactobacillus plantarum* (Group 1) and untreated fish samples (Group 3). The group 3 sample had the highest amount of histamine in all the temperature ranges tested. In all the groups tested, the low temperatures (0 and 5 °C) had very good preservative effect in terms of low levels of histamine production, as compared to high temperatures (15 and 25 °C) where the histamine content increased with increasing time of incubation (Table 1-4).

Table 1: Preservation of tuna fish treated with bacteriocin at 0 °C for different incubation period.

Group	Histamine content (mg/100 g) at different time of preservation			
	24 h	48 h	72 h	96 h
1	0.45±0.03^a	0.60±0.04^a	0.90±0.07^a	1.20±0.11^a
2	0.40±0.03^a	0.50±0.04^a	0.70±0.06^b	0.95±0.08^b
3	0.60±0.05^b	0.90±0.06^b	1.55±0.12^c	2.35±0.22^c

Table 2: Preservation of tuna fish treated with bacteriocin at 5 °C for different incubation period.

Group	Histamine content (mg/100 g) at different time of preservation			
	24 h	48 h	72 h	96 h
1	0.80±0.05^a	1.15±0.12^a	1.80±0.15^a	2.20±0.16^a
2	0.60±0.04^b	0.95±0.06^b	1.30±0.11^b	1.85±0.17^b
3	0.90±0.07^c	1.45±0.13^c	2.25±0.21^c	3.35±0.31^c

Table 3: Preservation of tuna fish treated with bacteriocin at 15 °C for different incubation period.

Group	Histamine content (mg/100 g)			
	24 h	48 h	72 h	96 h
1	2.45±0.22[a]	3.85±0.35[a]	6.75±0.59[a]	9.20±0.91[a]
2	1.40±0.11[b]	2.30±0.20[b]	3.80±0.33[b]	5.85±0.41[b]
3	3.30±0.25[c]	6.70±0.51[c]	10.4±0.94[c]	14.8±1.31[c]

Table 4: Preservation of tuna fish treated with bacteriocin at 25 °C for different incubation period

Group	Histamine content (mg/100 g)			
	24 h	48 h	72 h	96 h
1	2.95±0.25[a]	6.80±0.61[a]	10.7±0.91[a]	13.6±1.21[a]
2	1.95±0.11[b]	4.65±0.42[b]	7.30±0.61[b]	9.90±0.61[b]
3	5.80±0.47[c]	11.7±1.01[c]	17.9±1.51[c]	24.0±2.11[c]

Values are mean± standard error from 3 replicates in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Food preservation is the process of treating and handling food through many methods to preserve the value of food. The main effort is to stop or greatly slow down spoilage to prevent food-borne illness. Looking back at recent progress in food biotechnology, it is a fact that the problem of food safety and security still remains to be solved. This was the major challenge of the 1990's to the food industry, food and biological scientists and legislating authorities alike. In spite of the introduction of modern technologies and safety concepts (e.g. HACCP) the reported number of food-borne illnesses and intoxications is still increasing. The use of chemical preservatives is regulated by maximum permitted levels. These amounts vary between countries. Processors should check with their local authorities for the local regulations and for the regulations in the country of sale. Chemical preservatives cannot be used to cover up for poor quality raw materials. They are only added as a precaution to extend the shelf life of products by inhibiting microbial spoilage. Some chemical preservatives can taint the flavor of fruit juices if the recommended level is exceeded. Some consumers prefer to consume fruit juices with no chemical additives. On the other hand, an increasing number of consumers prefer minimally processed or prepared without chemical preservatives, as well as 'mild' and 'light' products characterized by a low acid, sugar or fat content. Many of these 'ready-to eat' and novel food types represent new food systems with respect to health risks and food spoilage. Against this background and relying on improved understanding and knowledge of the complexity of microbial interactions and combined preservation factors present in the food systems, recent approaches are increasingly directed towards possibilities offered by biological or 'milder' preservation approaches. This implies so-called 'protective cultures' or theirs metabolites, notably enzymes and bacteriocins (Leal-Sanchez *et al.,* 2002).

The potential use of bacteriocin-producing *lactobacilli* as bio-preservatives requires good competitive properties of the added culture with the indigenous microflora in the specific product, yet not in themselves increasing the spoilage rate of the product. Furthermore, a complete understanding of the influence of the intrinsic and extrinsic conditions to be found in the food matrix on the production of bacteriocin is important for their commercial application as protective cultures in fish preservation. In this respect, bacteriocin titres can dramatically change on altering environmental conditions and optimum production may require a certain combination of influencing factors (Haaland *et al.,* 1990**).** Regarding the complexity of food environments, a better knowledge of interactions of these factors on bacteriocin production is needed.

The most important factor that contributes to the production of toxic biogenic amines during post-harvest handling is the storage time and temperature. Both the post-mortem formation of amino acids and their rapid decarboxylation are temperature-dependent (Baranowski *et al.,* 1990). Temperature-abuse potentiates histamine formation in fresh fish (*Coryphaena hippurus*) at 32 °C is reportedly increasing from 1.6 to 2,920 ppm in 24 h. High histamine formers producing >1,000 ppm histamine were mostly isolated from fish stored at 15-25°C (Kim *et al.,* 2000).The present experiment revealed that there was an increasing trend of histamine production within 24 h of exposure and significant amount of histamine was also increased with increasing time and temperatures (Table 1-4).

Kim *et al.* (2000) have identified the bacterium producing the highest level of histamine when fish are exposed at 25 °C rather than low temperatures. The isolate produces the highest level of histamine that is 5253 ppm at 25 °C in the stationary phase of its growth. At 15 °C, histamine production is reduced to 2769 ppm. Neither microbial growth nor histamine formation is detected at 4 °C. In the present study also, a similar type of results was observed (Table 1-4).

There is no good agreement in the world as to what level of histamine accumulation represents the toxic level. As multiple factors are involved with human diet the problem is not a simple one to establish. The most generally accepted level for the accumulation of histamine in fish or fish products is 10 mg % (meaning 10 mg of free histamine per 100 g sample). Beyond that level the fish is considered to have spoiled even though it may not be obviously toxic (Arnold *et al.,* 1980). However, it is not unusual to hear of toxic reactions, which appear to be histamine poisoning, to much lower concentrations. In the present study, group 2 (fish samples preserved with

partially purified bacteriocin) exhibited the accepted histamine level (9.90 mg %), whereas the other two groups – group 1 (fish samples preserved with crude bacteriocin) and group 3 (unpreserved fish sample) - failed to control the production of histamine beyond the limit (Table 1-4).

CONCLUSION

It was concluded that isolates of histamine-producing bacteria from a local tuna fish and to test antibacterial activity of mangrove isolates of *Lactobacillus* species against the histamine producing bacteria. According to the results obtained it was proved that mangrove isolates of *Lactobacillus* species having high antimicrobial activity against histamine-producing bacteria which is responsible for spoilage of sea foods.

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