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Study of Histamine Forming Bacteria in Commercial fish samples of Kalyan city

PA Joshi*, Vishal S. Bhoir

Department of Microbiology, Birla College, Kalyan, Maharashtra 421304 , India

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ABSTRACT

Histamine food poisoning is found to be associated with consumption of scombroid fish containing unusually high levels of histamine. Fish belonging to non-scombroid group may also cause histamine poisoning. In this study, histamine forming bacteria in the commercial fish samples of local markets of Kalyan region were investigated. Among 54 isolates 24 were found to be prominent histamine producers. A simple and rapid colorimetric method for the quantification of histamine in fish was used. Histamine level in fresh mackerel samples was found to be around 20 mg/100 g, which was much above the defect action level (5 mg/100 g) given by FDA indicating potential risk for histamine poisoning. The study suggest that practice of more hygienic and sanitary conditions during handling and processing of fish are required to minimize the contamination of such histamine producing bacteria.

1. Introduction

Histamine is a biogenic amine produced during microbial decomposition of scombroid fish such as mackerel as well as non scombroid fish such as sardines. Consumption of spoiled fish results in the outbreaks of food poisoning and histamine fish poisoning is one such type of food poisonings [1]. Scombroid poisoning is usually a mild illness with a variety of symptoms including rash, urticeria, nausea, vomiting, diarrhea, flushing, tingling and itching of the skin [2].

Scombroid fish poisoning results from eating the spoiled fishes of family Scombroidae. These fish contain characteristically high level of free histidine in their muscle tissue, which will be converted to histamine under conditions favorable to bacterial growth and synthesis of histidine decarboxylase [3,4].Scombroid fishes include tuna, mackerel, skipjack and bonito. However non scombroid fishes such as mahi-mahi, blue fish, amberjack, herrings, sardines and anchovies have also been implicated in histamine fish poisonin [1].

* Corresponding Author : Dr. (Mrs). P.A. Joshi,
Head of Department of Microbiology,
Birla College, Kalyan, Maharashtra 421304 , India.
Mob: 9833154515.
Email: pradnyajoi@gmail.com,

The formation of histamine in scombroid and other marine fish containing abundant endogenous histidine has been attributed to microbial action rather than to endogenous histidine decarboxylase activity [5,6]. The histidine can be catabolized by two ways in fish muscle. One is by histidine deamination to obtain urocanic acid and other by histidine decarboxylation to form histamine [7]. The deamination activity is the principal way in normal physiological conditions; decarboxylation activity can be most important in other circumstances, e.g. bacterial contamination [8]. Several studies of the normal microbial population of marine fish revealed their ability to produce high amounts of histamine at low temperatures [5,9,6,10].

The tropical climate of India with an average temperature ranging between 25-40°C is suitable for proliferation of histamine forming bacteria in fish and fish products. Various stages of fish handling (harvest, procurement, retail marketing) and processing (drying, salting, freezing) have profound effect on histamine formation. Majority of fishes are salted and dried without removing gut which harbor large number of bacteria that possess the decarboxylase enzyme [11]. The purpose of this study was to isolate, detect histamine forming bacteria and quantification of histamine in fresh and salted fish products available to the local markets in kalyan region.

2. Material and methods

Fresh fish viz. Indian mackerel (Rastrelliger kanagurta) and sardines (Sardinella gibbosa) as well as salted mackerel and sardines were purchased from the retail shops at Kalyan. Samples were brought to laboratory and subjected to microbiological and colorimetric analysis within an hour.

Fish muscle tissue (5 g) was obtained from each of three locations (head, belly and tail) and transferred to 50 ml of 0.85% NaCl solution (saline). The sample was homogenized for 2 min using high speed blender and centrifuged at 4000 rpm for 10 min. The supernatant was made up to 25 ml with saline. The muscle extract was used immediately for histamine analysis. A composite tissue sample was prepared by aseptically sampling 5 g from each location, diluting 1:10 (wt/vol) with sterile 0.1 % peptone water, homogenized using high speed blender. Immediately after blending samples were used for microbiological analysis.

Fish composite samples were serially diluted in sterile 0.1% peptone water and 0.1 ml were spread plated in duplicate on tryptic soya agar (TSA) (HiMedia, Mumbai, India). For salted fish tryptic soya agar with 7.5% NaCl was used and incubated at 37˚C for 48 h. Representative isolates were selected from TSA plate. Isolates were purified by sequential streaking on TSA plates and incubation at 37˚C for 48 h.

The pure cultures were transferred to TSA slants containing 2% NaCl and incubated at 37˚C for 24 h. Each isolate taken from each slant was plated on Niven's agar medium and incubated at 37˚C for 48 h to screen for histamine production. Niven's positive isolates were Gram stained and examined under oil immersion. All isolates were further identified using biochemical tests according to Bergey's manual [12].

Quantification of histamine was carried out using colorimetric method reported by Patange et al [13]. In this method, 1 ml of the muscle extract was taken into a glass-stoppered test tube and diluted to 2 ml with saline and 0.5 g of salt mixture containing 6.25 g of anhydrous sodium sulfate to 1 g trisodium phosphate monohydrate was added. The tubes were stoppered and shaken thoroughly. 2 ml of n-butanol was then added and the tubes shaken vigorously for 1 min and allowed to stand for 2 min and then shaken briefly to break the protein gel. The tubes were further shaken vigorously for few seconds and then centrifuged at 3100 rpm for 10 min. The upper butanol layer (only 1 ml) was transferred into a clean and dry test tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml of distilled water. In a clean tube 5 ml of 1.1% sodium carbonate solution was taken and 2 ml of the chilled reagent, p-phenyldiazonium sulfonate 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 color produced was measured immediately after 5 min at 496 nm. The concentration of histamine in sample was obtained from the standard curve for the corresponding absorbance measured at 496 nm. The histamine concentration in sample was estimated using the following formula.

$$\text{Histamine (mg/100 g)} = \frac{A \times 2 \times 25 \times 100}{5 \times 1000} = A \text{ mg/100 g}$$

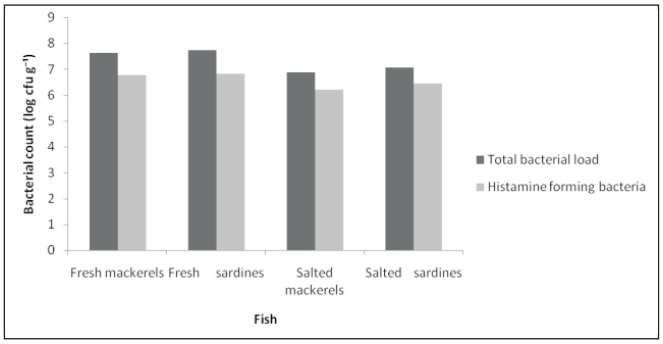
where A is the value of histamine obtained in l g/ml from the standard curve.

For histamine confirmation Gram positive and Gram negative isolates were streaked in triplicates on TSA plates containing 2% NaCl supplemented with 2% histidine and incubated at 37˚C for 24 h. A representative colony from each of the plate was inoculated into 9 ml of tryptic soy broth supplemented with 2% NaCl, 2% histidine and 0.0005% pyridoxal-HCl (pH 5.8) [TSB+] and incubated at 37˚C for 24h. A 1 ml sample of each TSB+ suspension was transferred into a tube of fresh TSB+ media and incubated at 37˚C for 48 h. A 3 ml subsample of this final culture was transferred into polypropylene centrifuge tubes and centrifuged at 1170 rpm for 20 min. Supernatants were diluted 1 to 10 in saline and histamine concentrations were determined with colorimetric assay of histamine [13].

3. Results

The counts of total bacteria and histamine forming bacteria found in the commercial fish samples are presented in Fig 1. Figure shows that in case of both fresh mackerel and fresh sardines the total bacterial as well as histamine forming bacterial counts were similar while in case of salted sardines both total bacterial and histamine forming bacterial counts obtained were higher than salted mackerels. The total average bacterial load of fresh fish was 105 cfu/g. The histamine forming bacteria in fresh fish was one log lower than the total bacterial load. The total average bacterial load of salted fish was 104 cfu/g and the total histamine forming bacteria was one log lower than the total bacterial load.

Fig 1. Total bacterial load and histamine forming bacteria in fish samples



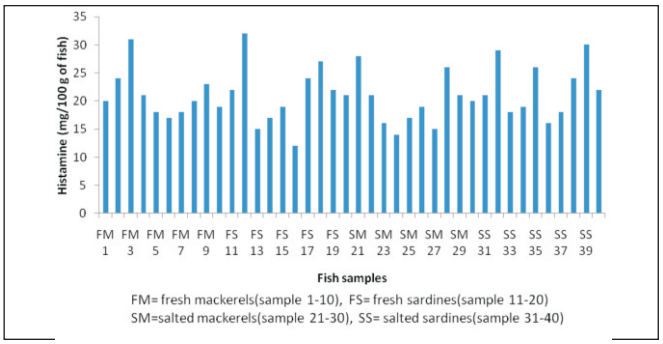
In the present study, 15 strains were isolated from fresh mackerel, 13 strains were from salted mackerel, 14 strains were from fresh sardines and 12 strains were from salted sardines, in a total 54 strains were isolated. All these strains were preliminarily investigated for their ability to produce histamine in Niven's medium and among them 24 strains showed positive results on Niven's medium.

Among the 24 Niven's positive isolates, 17 isolates were identified as Gram negative rods and 7 were identified as Gram positive rods or cocci.

Biochemical analysis revealed that histamine producing bacteria obtained in this study belonged to following genera viz. Bacillus, Pseudomonas, Vibrio, Staphylococcus, Morganella, Enterobacter and Klebsiella [12].

The 24 Niven's positive isolates were tested to verify their ability to produce histamine. Only 7 were confirmed as histamine producer as determined by colorimetric assay of histamine. In this study a total of 40 samples were tested, 10 each of the fresh mackerel, fresh sardines, salted sardine and salted mackerel (Fig. 2).Figure shows that from 40 samples, 4 of fresh mackerels, 3 of fresh sardines, 4 of salted mackerels and 4 of salted sardines had given histamine level≥20 mg/100g.

Fig 2. Histamine concentrations of fish samples



bacteria obtained in this study was high as compared to other studies reported [11,17]in which histamine forming bacterial counts were 2-3 log lower than total bacterial load.

In addition to scombroid and non scombroid fish histamine producing bacteria are also found in other food such as meat and meat products [18,19,20], cheeses [21,22,23], some fermented products [24] and some beverages [25,26,27].

Histidine decarboxylases are found in certain Enterobacteriaceae, Clostridium and Lactobacillus sp. [28]. Histamine producing bacteria obtained in this study were also reported in many other studies such as Morganella morganii [29,30], Klebsiella pneumoniae [31,32], Hafnia alvei [33], Proteus sp., Clostridium perfringens, Enterobacter aerogens, Vibrio alginolyticus [34,35,36], Bacillus sp. [37], histamine forming bacteria have been identified in halotolerent Staphylococcus, Vibrio and Pseudomonas [38,39]. Psychrophilic and mesophilic halophilic histamine forming bacteria have been isolated from marine fish [40,41,42].

The Niven's method is based upon a pH shift in media that can lead to false positive results due to change in pH by metabolic process of microorganisms [43,44]. The low percentage (29%) of confirmed histamine producer was consistent with other studies of Niven's positive isolates in which histamine confirmation by quantification methods confirmed that only 15 to 37% of isolates were actually histamine producers [45,46,47,48]. The confirmation of histamine producing bacteria demonstrates the potential risk for contamination of fish with these bacteria [44].

Factors affecting growth of histamine producing bacteria include type and size of fish, handling techniques and cooling methods (FDA, 2001). In this study, most of the fishes in the markets were kept outside the ice vessels for sell for considerable amount of time which results in ambient temperature abuse of fish and this gives opportunity for histamine producing bacteria to proliferate.

The FDA guidelines for tuna, mahi-mahi and related fish specified 5 mg/100 g as defect action level [13] and ≥50 mg/100 g as toxicity level [49,13]. The European Economic Community (ECC) has recently established regulation for species of fish belonging to the Scombridae and Clupedae families and fixed a three-class plan for maximum allowable levels of histamine in fresh fish (n=9; c=2; m = 100 ppm; M = 200 ppm) and enzymatically ripened fish products (n = 9; c = 2; m = 200 ppm; M = 400 ppm) where n is the number of units to be analyzed from each lot, m and M are the histamine tolerances, and c is the number of units allowed to contain a histamine level higher than m but lower than M [50].

5. Conclusion

In this study, the histamine level obtained was in between 20-30 mg/100 g which was much above the defect action level and commercial fish samples had considerable incidence of histamine producing bacteria which on proliferation under suitable condition may contribute to toxic histamine accumulation.

The study revealed the incidence of histamine producing bacteria in all fish samples and their incidence can be minimized by implementation of more hygienic and sanitary conditions during handling and processing of fish as per the guidelines of FDA.

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