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RESEARCH ARTICLE

Isolation and identification of histamine-producing bacteria in frigate tuna *Auxis thazard*

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ABSTRACT

Histamine is the most common cause of human foodborne illness and is found to be associated with the consumption of fish products especially in scombroid fishes containing unusual levels of histamine. In this study, histamine-producing bacteria in the Frigate Tuna, *Auxis thazard* samples of the local Ukkadam fish market of Coimbatore were investigated. Among 12 isolates 4 were found to be prominent histamine producing bacteria. An automated microbiology system called VITEK, a biochemical test analyzer was used for the identification of bacteria, and four isolates were tentatively identified as *Sphingomonas paucimobilis*; *Proteus vulgaris*; *Providencia rustigianii*, and *Neisseria zoodegmatis*. The isolation of histamine-producing bacteria from the muscle emphasizes the possible importance of muscle as a reservoir for bacterial contamination. The study suggests that the practice of more hygienic and sanitary conditions during the handling and processing of fish is required to minimize the contamination of such histamine-producing bacteria.

Keywords: Frigate Tuna, Histamine-producing bacteria, *Proteus vulgaris*, VITEK, Coimbatore

1. INTRODUCTION

Histamine poisoning is an allergy-like intoxication most frequently associated with the ingestion of various types of fermented foods, including meat [1], dairy [2], soybean products [3], beer [4], vegetables [5] including fish [6]. The fishes from the families Scomberesocidae and Scombridae, such as tuna, skipjack, mackerel, and bonito. However, scombroid fish poisoning is a bit of a misnomer because certain types of non scombroid fish are also commonly involved, including mahi-mahi, bluefish, jack mackerel, sardines, yellowtail, anchovies, and herring. Consumption of spoiled fish results in the outbreak of food poisoning and histamine fish poisoning is one such type of food poisoning [7]. Histamine is a chemical hazard and it is the postmortem product in fish muscle, but it can exist in fresh fishes at a very low level, improper handling and storage of fishes can increase histamine formation by the proliferation of bacteria possessing histidine decarboxylase [8]. Scombroid fish poisoning results from eating the spoiled fish of the family Scombridae. These fishes contain a characteristically high amount of free histidine in their muscle tissue, which will be converted to histamine under favorable conditions for the bacterial growth and synthesis of histidine decarboxylase [9]. The tropical climate of India with an average temperature ranging between 25-40˚C is suitable for the proliferation of histamine-forming bacteria in fish and fishery products. Various stages of fish handling (harvest, procurement, retail marketing) and processing (drying salting, freezing) have profound effects on histamine formation [10].

The allergic effects of scombroid toxin (histamine) from fish poisoning usually appear a few minutes to a few hours after a person has ingested histamine-containing fish and affect various organ systems, which leads to the occurrence of various intestinal and extra-intestinal symptoms [11]. Cheese can also occasionally be involved, especially Swiss cheese [12]. Histamine poisoning occurs on a worldwide scale with several hundred cases reported annually to public health agencies. The toxin (histamine) is created within the flesh of certain fish under specific environmental conditions. Histidine is present in the muscle protein of Scombridae. In the presence of certain bacteria, histidine gets broken down to histamine. Ingestion of the flesh of improperly handled fish causes ingestion of large amounts of histamine and the development of symptoms of histamine poisoning. Histamine is not destroyed or inactivated by heating or cooking. Contaminated fish often looks and smells

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normal, but is periodically described as having a peppery taste. Proper refrigeration/freezing of fresh fish will dramatically reduce the risk of scombroid poisoning. Pure histamine taken orally is substantially metabolized in crossing the intestinal wall or in the liver and produces only mild symptoms at relatively high doses [13].

The purpose of this study was to isolate, detect, and identify the histamine-producing bacteria in frozen tuna fish collected from the local Ukkadam fish market of Coimbatore.

2. MATERIALS AND METHODS

2.1. Sample collection

Frozen fish sample the Frigate Tuna, *Auxis thazard* collected from the fish market in Ukkadam, Coimbatore. The fish was frozen in a deep freezer at 5˚C and delivered to the laboratory where analysis of bacterial counts, isolation of Histamine Producing Bacteria (HPB), and histamine content was carried out after 48 h. Fish muscle tissue (5g) was taken from the belly region and transferred to 50ml of 0.85% sodium chloride solution (Saline). The sample was homogenized using mortar and pestle and centrifuged at 4000 rpm for 10 min. The supernatant was made up to 25ml with saline water. The muscle extract was used immediately for bacterial analysis.

Systematic position

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Scombriformes
Family: Scombridae
Genus: *Auxis*
Species: *A. thazard* (Lacepede, 1800)

Figure. 1. *Auxis thazard*

2.2. Identification of histamine producing bacteria (HPB) using niven agar

Fish composite samples were serially diluted in sterile 0.1% peptone water and 0.1 ml were spread plated on trypticase soya agar (TSA) in duplicate 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 incubated at 37˚C for 48 h. The pure cultures were kept in TSA slants containing 2% sodium chloride and incubated at 37˚C for 24 h. Each isolate taken from each slant was plated on modified Niven's agar medium and incubated at 37˚C for 48 h to screen for histamine production and it was observed based on the pink halo glowing action (pigmentation) [14].

For the presence of histamine confirmation, gram-positive and gram-negative isolates were streaked in triplicates on TSA plates containing 2% sodium chloride supplemented with 2% histidine and incubated at 37˚C for 24 h.

The identification of 4 colonies of bacteria from the selected isolates was done by VITEK, a biochemical test analyzer. VITEK is an automated microbiology system utilizing growth-based technology. This system accommodates colorimetric reagent cards that are incubated and interpreted automatically. Bacterial identification through VITEK has more accuracy [16].

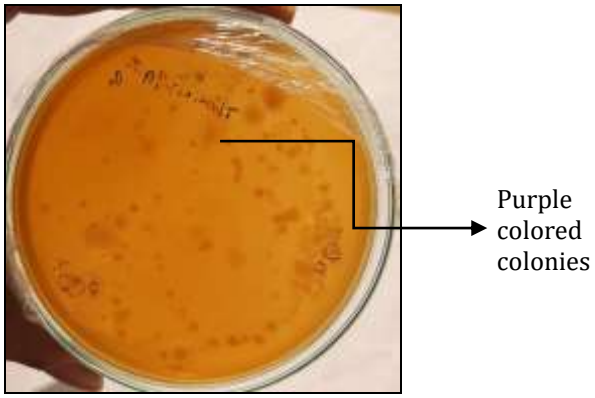

Figure 2. Histamine-producing bacteria in modified Niven Agar medium

3. RESULT AND DISCUSSION

The presence of strong microbial activity was noted at 12×10^{-6} CFU/ml whereas the presence of histamine-producing bacteria was much lower than the total bacterial load. In the present study, 12 colonies were selected based on the appearance of purple-colored colony in the Niven agar medium. For pure culture, 4 colonies were sub-cultured and used for further characterization.

Table 1. Biochemical characteristics of bacterial colonies isolated from the muscle of Frigate Tuna, *Auxis thazard*.

Biochemical Test	Frigate Tuna (*Auxis thazard*)			
	Colony1	Colony2	Colony3	Colony4
Ala-Phe-Pro-ARYLAMIDASE	-	-	-	-
L-Pyrrolydonyl-ARYLAMIDASE	-	-	-	-
BETA-GALACTOSIDASE	-	-	-	-
BETA-N-ACETYL-GLUCOSAMINIDASE	-	-	-	-
Glutamyl Arylamidase pNA	-	-	-	-
GAMMA-GLUTAMYL-TRANSFERASE	+	-	-	+
BETA-GLUCOSIDASE	-	+	-	-
BETA-XYLOSIDASE	+	-	-	+
BETA-Alanine arylamidase pNA	-	-	-	-
L-Proline ARYLAMIDASE	-	+	+	-
LIPASE	-	+	+	-
Tyrosine ARYLAMIDASE	-	+	+	-
UREASE	-	-	-	-
CITRATE (SODIUM)	-	+	-	-
MALONATE	-	-	-	-
5-KETO-D-GLUCONATE	-	-	+	-
L-LACTATE alkalinization	-	-	-	-
ALPHA-GLUCOSIDASE	-	-	-	-
SUCCINATE alkalinization	+	-	-	+
Beta-N-ACETYL-GALACTOSAMINIDASE	-	-	-	-
ALPHA-GALACTOSIDASE	-	-	-	-
PHOSPHATASE	-	+	+	-
Glycine ARYLAMIDASE	-	+	-	-
ORNITHINE DECARBOXYLASE	-	-	-	-
LYSINE DECARBOXYLASE	-	+	-	-
COUMARATE	-	-	-	-

BETA GLUCORONIDASE	-	-	-	-
Glu-Gly-Arg-ARYLAMIDASE	-	-	-	-
L-LACTATE assimilation	-	-	-	-
H2S PRODUCTION	-	-	-	-
L-HISTIDINE assimilation	-	+	-	-
O/129 RESISTANCE	-	-	-	-
L-MALATE assimilation	-	+	-	-
ELLMAN	-	-	-	-

Table 2. Biochemical tests for carbohydrate fermentation by bacterial colonies isolated from Frigate Tuna, *Auxis thazard*.

Biochemical Test	**Frigate Tuna (*Auxis thazard*)**			
	Colony 1	Colony 2	Colony 3	Colony 4
ADONITOL	-	-	-	-
L-ARABITOL	-	-	-	-
D-CELLOBIOSE	-	-	-	-
D-GLUCOSE	-	-	-	-
FERMENTATION/ GLUCOSE	-	+	+	-
D-MALTOSE	-	-	-	-
D-MANNITOL	-	-	-	-
D-MANNOSE	-	-	-	-
PALATINOSE	-	-	-	-
D-SORBITOL	+	+	+	+
SACCHAROSE/SUCROSE	-	-	-	-

+ ⟶ Positive result
- ⟶ Negative result

Table 3. Identification of Histamine Producing Bacteria by using VITEK, biochemical test analyzer

Colony 1	*Sphingomonas paucimobilis*
Colony 2	*Proteus vulgaris*
Colony 3	*Providencia rustigianii*
Colony 4	*Neisseria zoodegmatis*

The presence of histamine-producing bacteria in the collected fish samples has been isolated by using the modified Niven Agar (pH 6.5) [14], in which histamine-producing bacteria grew in the purple-colored colony. 12 Different colonies were identified on the basis of the morphology. Because the histamine-producing bacteria are highly resistant to acidity in nature and most of them are halophiles, mesophiles, and psychrotolerants. The mesophilic bacteria can grow well in frozen as well as refrigerated conditions [15].

Biochemical analysis for the identification of histamine-producing bacteria was done by VITEK, a biochemical test analyzer. The enzyme test of tuna showing colonies 1, 2, 3, and 4 show negative results for the Ellman test. Colonies 2 and 3 show a positive response since they produce an enzyme called tyrosine arylamidase and show negative results in the O/129 Resistance test. Colony 1 was able to produce enzymes such as gamma-glutamyl-transferase and beta-xylosidase. Colony 2 produced different kinds of enzymes like beta-glucosidase, L-proline arylamidase, lipase, tyrosine arylamidase, phosphatase, glycine arylamidase, lysine decarboxylase. Likewise, colony 3 also produced different kinds of enzymes such as L-proline arylamidase, Lipase, Tyrosine arylamidase, 5-keto-d-gluconate, and phosphatase. Furthermore, colony 4 produced different kinds of enzymes such as gamma-glutamyl-transferase and beta-xylosidase (Table 1).

The carbohydrate fermentation activity of the isolates of tuna details that colony 1 and 4 reduces the sugar called D-Sorbitol, whereas colony 2 and 3 reduces the sugar called D-Sorbitol and Glucose (Table 2).

The identified histamine producing bacteria are *Sphingomonas paucimobilis, Proteus vulgaris, Providencia rustigianii, Neisseria zoodegmatis* by using VITEK, a biochemical test analyzer (Table 3).

4. CONCLUSION

The study revealed the presence of histamine-producing bacteria in the tuna fish sample and their incidence can be minimized by the implementation of more hygienic and sanitary conditions during handling and processing of fish as per the guidelines of FDA. Through this study, the consumers of frozen Frigate Tuna, *Auxis thazard*, sold from the Ukkadam Fish Market, Coimbatore should check out the quality of tuna fish. The isolation of histamine-producing bacteria from the muscle emphasizes the possible importance of the muscle as a reservoir for these bacterial contaminations.

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