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Research Article

A PILOT STUDY ON THE ISOLATION AND IDENTIFICATION OF HISTAMINE-PRODUCING BACTERIA FROM NARROW BARRED SPANISH MACKEREL AND JAPANESE THREAD FIN BREAM FROM UKKADAM FISH MARKET, COIMBATORE, INDIA

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ABSTRACT

Consumption of marine fishes with high histamine levels due to improper storing leads to cause scombroid or histamine fish poisoning. In spoiled fishes, bacteria begin to break down the histidine into histamine by decarboxylation activity. Previous studies reported that black muscle fishes usually produce large amounts of histamine-producing bacteria (HPB). In this study, two white muscle fishes such as *Scomberomorus commerson* (Narrow-barred Spanish mackerel) and *Nemipterus japonicus* (Japanese threadfin bream) were chosen to detect the presence of histamine-producing bacteria (HPB). The fish samples were purchased from Ukkadam fish market in Coimbatore and examined for the occurrence of HPB on Niven agar. The isolated HPB on media were analyzed for identification based on their morphological characteristics, primary test, and biochemical test. Finally, 8 colonies were confirmed as the cause for histamine production of both fishes and among them, 5 belong to the family *Enterobacteriaceae* and others belong to the family *Aeromonadaceae, Morganellaceae,* and *Enterococcaceae*. Our study showed that the histamine-producing bacteria highly reported in dark-muscle fishes were also isolated from the chosen white-muscle fishes. Hence the presence of HPB indicates the improper maintenance of fish in retail fish markets. This considerable incidence of histamine-producing bacteria which on proliferation under suitable conditions may contribute to toxic histamine accumulation in the flesh of fishes.

Keywords: Histamine-producing bacteria, Niven agar, *Nemipterus japonicus*, *Scomberomorus commerson.*

INTRODUCTION

Fish products constitute an important part of the human diet because they are an excellent source of nutrients, including proteins, vitamins, salt minerals, and polyunsaturated fatty acids (Visciano, 2015). Due to this high value of nutrient content, this product is easily contaminated by bacterial species, as it is used as an energy source by microbial contaminants (Jaaskelainen *et al.*, 2018), and (Kartikaningsih *et al.,* 2021). The inappropriate storage of fish and temperature abuse can lead to biogenic amine formation due to microbial enzymatic activities. Gram-positive and negative bacteria associated with fish spoilage can produce biogenic amines that can spread to muscle tissue (Visciano *et al.,* 2020 and FAO/WHO 2012). The occurrence of biogenic amines in fish is directly associated with microorganisms with decarboxylase activity. Histamine, tyramine, putrescine, and cadaverine are the most common biogenic amines found in fish and derive from the decarboxylation of corresponding free amino acids by microorganisms (Pester 2011).

Histamine (2-[4-Imidozolyl] ethylamine) was discovered by Dale HD and Laidlaw (1910) and it was identified as a mediator of anaphylactic reactions by Steinhoff (1932). Histamine is a naturally occurring organic nitrogenous compound in mammalian physiology. It is present in mast cells and basophils, its biological effect is seen only when it is released in large amounts in the course of an allergic reaction (Cavanah *et al.* 1993).Histamine has

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been shown to have a variety of functions in neurotransmission, immunomodulation, hematopoiesis, wound healing, day-night rhythm, and the control of histamine and polyamine-induced cell proliferation and angiogenesis in tumor models (Raithel *et al.*1998 and Kusche *et al.* 1980). Histidine decarboxylase has an important role in the histamine synthesis pathway, producing histamine in a one-step reaction. Histamine cannot be generated by any other known enzyme (Shahid and Mohammad, 2009). This enzyme utilizes a pyridoxal 5'-phosphate (PLP) as a cofactor (Riley and Snell, 1968) and Rosenthaler *et al.* 1965). In addition to the mammalian cells, different bacterial strains have been identified as being able to secrete histamine following decarboxylation of histidine. Bacteria containing histidine decarboxylase are the main source of histamine formation in scombroid fish. This enzyme is responsible for the conversion of free histidine in fish muscle to histamine, commonly known as the scombroidtoxin. The Scombroid toxin has been described highly in the family Scombridae such as tuna, mackerel, skip jack, and bonito; it has also been described with other dark fleshed fish as mahi-mahi, bluefish, amberjack, herring, sardines, and anchovies. Histamine is heat-resistant and, hence can't be removed by cooking, freezing, canning, or smoking (Hungerford (2010), Jantschitsch (2011). Symptoms of scombroid fish poisoning include headache, dizziness, rashes, nausea, vomiting, diarrhea and sometimes swelling of the face and tongue. Plasma histamine accumulation can provoke a wide number of nonspecific gastrointestinal and extra intestinal clinical manifestations such as dermatological, respiratory, neurological and hemodynamic complaints (Comas-Basté *et al.* 2020, Kovacova-Hanuskova *et al.* 2015, Maintz and Novak, 2007). The most frequent and severe symptoms, according to a recent comprehensive study, were abdominal distension, diarrhea, postprandial fullness, abdominal pain, and constipation, followed by headaches, dizziness, and palpitations (Schnedl *et al.* 2019).

Scomberomorus commerson

Nemipterus japonicus

Figure 1.Fish samples purchased from Ukkadam fish market Coimbatore.

So far studies have been focused on histamine-producing bacterial identification in fish sample. Such as the most commonly isolated HPB-causing scombroid toxins were *Morganella morganii, Klebsiella pneumoniae,* and *Hafnia alvei* (Taylor *et al.*1986, Rawles *et al.* 1996). Later, *Enteric bacteria* were reported as the most important HPB, and *M. morganii was* recognized as the high histamine former both in fish and culture broth (Klausen *et al.*1987, Ababouch *et al.* 1991, Lopez-Sabatier *et al.* 1994). *Proteus vulgaris, Proteus mirabilis, Enterobacter aerogenes, Enterobacter cloacae, Serratia fonticola, Serratia liquefaciens,* and *Citrobacter freundii* were also isolated as histamine producers in fish (Lopez-Sabater *et al.*1996). To the best of our knowledge, there are no studies have been done on Narrow-barred Spanish mackerel and Japanese threadfin bream collected from Ukkadam fish markets. In this present study, we have approached this in detail by doing bacterial identification from these two species collected from the Ukkadam fish market, in Coimbatore. The reason behind the presence of histamine poisoning is the consumption of fresh, canned, or smoked fish with high levels of histamine due to improper processing or storage.

MATERIALS AND METHODS

Sample collection

Dead marine fishes such as *Scomberomorus commerson* (Narrow-barred Spanish mackerel) and *Nemipterus japonicus* (Japanese threadfin bream) were purchased from the Ukkadam fish market, Coimbatore (Figure 1). Fish muscles near the gills were collected and weighed at 50g. The samples were frozen and thawed for one day and used for further studies.

Identification of the presence of Histamine Producing Bacteria (HPB)

The thawed muscle sample was homogenized with 0.1% peptone water in the dilution of 1:3 (wt/vol) aseptically by using a mortar and pestle. This mixture was centrifuged at 4000 rpm for 10 min to collect the supernatant. 1ml of

sample supernatant was serially diluted in 9 ml of tryptic soy broth (TSB) to 6 dilutions and spread onto Niven agar (NA), incubated at 30^{o} C for 5 days (Joshi *et al.* 2011).

Niven media

The Niven media was prepared as 0.5% tryptone, 0.5% yeast extract, 2% L-histidine monohydrochloride, 0.5% NaCl, 0.1% $CaCO_3$, 2% agar, and 0.006% bromocresol purple were dissolved in the distilled water and adjusted the pH to 6.5 (Niven *et al*. 1981; Yoshinaga and Frank, 1982). The NA plates are routinely examined for the occurrence of purple halo bacteria (Niven *et al.* 1981). The positive purple halo bacteria were isolated and streaked on tryptic soy agar (TSA) to secure the pure culture. Duplicates of slant culture for these positive isolates were prepared and stored in the refrigerator. Primarily these strains were tested for gram staining and endospore staining, motility and also analyzed morphologically to isolate the similar colonies separately. Then, the species identification of positive isolates was done by VITEK, a biochemical test analyzer. VITEK is an automated microbiology system utilizing growth-based technology that supports Colorimetric reagent cards that are incubated and analyzed automatically. Bacterial identification through VITEK has more accuracy (Guo L *et al*. 2014).

RESULTS AND DISCUSSION

In order to detect the microbes from Narrow barred Spanish mackerel (*Scomberomorus commerson*) and Japanese threadfin bream (*Nemipterus japonicus*), the spread plate of Niven agar was analyzed Histidine decarboxylating colonies generally appeared on Niven agar as purple colonies with a purple halo on the yellow background (Figure 2). Colonies obtained on these plates were expressed by CFU (Colony Forming Unit) for the total bacterial count. CFU of both fish samples such as Mackerel and Threadfin bream were compared using log CFU/ml formula (Figure 3). The data showed that an acceptable number of colonies were observed in the dilution of 10^{-5} of both fishes. The total bacterial populations were 225×10^{-5} CFU in mackerel and 180×10^{-5} CFU in threadfin bream (Table 1).

The CFU of HPB was obtained as 45×10^{-5} CFU in mackerel and 28×10^{-5} in threadfin bream. In this study, 12 strains of mackerel and 9 strains of thread fin bream were isolated from the niven agar. Of the total 21 bacterial colonies, only 4 strains of mackerel and 4 strains of thread fin bream were isolated as histamine-producing colonies by their distinct morphological characteristics such as color, growing pattern, and size of the colony. In the primary test, all 8 colonies appeared to be motile and vegetative cells, as they don't produce endo-spores. Inmackerel, all 4 colonies were gram-negative. In thread fin bream, the colony 1, 2, and 3 are gram-negative and colony 4 is observed as gram - positive (Tables 2 - 3).

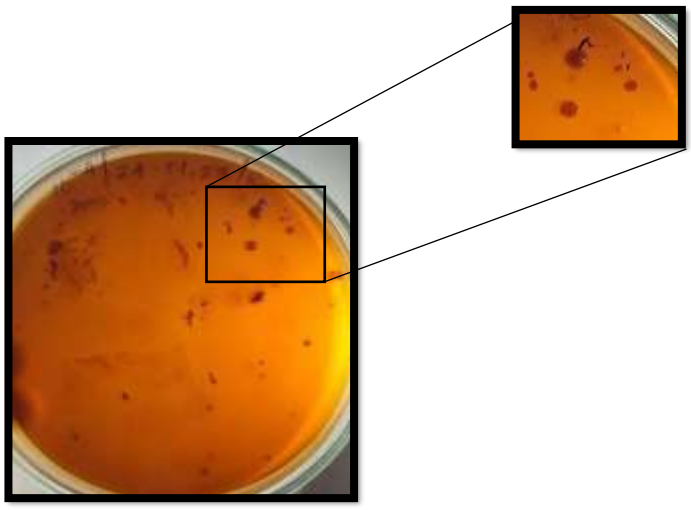

Figure 2.The marked colony showing the presence of purple halo bacteria growth in Niven agar medium.

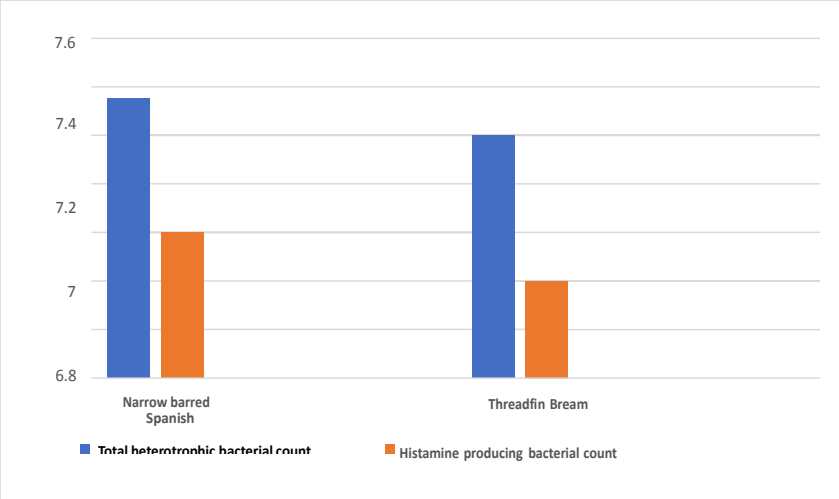

Figure 3.The total average bacterial load and total histamine-producing bacterial load represented as (LogCFU/ml).

The biochemical properties of these 8 colonies were analyzed and identified by VITEK an, automated microbiology system. The enzyme test of bacterial isolates from mackerel is presented in Table. Colonies1, 2, and 3, resulted positive to ellmantest.Colonies1, 2, and 3 produced tyrosine arylamidase and showed positive results on the O/129 resistance test. Colony 1 was able to produce enzymes such as L-pyrrolidinyl-arylamidase, beta-galactosidase, beta-N-acetyl-glucosaminidase, L-proline arylamidase, coumarate, glu-gly-arg-arylamidase. The enzymes identified to be present in colony 2 were urease, L-lactate alkalinization, phosphatase, and ornithine decarboxylase. Further, colony 3 showed positive results for the enzyme such as beta-galactosidase, beta-N-acetylglucosaminidase, gamma-glutamyl-transferase, L-proline arylamidase, citrate, L-lactate alkalinisation, succinate alkalinization, phosphatase, ornithine decarboxylase, lysine decarboxylase, L-lactate assimilation. Colony 4 produced enzymes like L-pyrrolidonyl - arylamidase, beta-galactosidase, urease, phosphatase, and coumarate and also observed H_2S production.

The carbohydrate fermentation activity of isolates from mackerel detailed that colonies1 and 4 reduce sugars like glucose and D-glucose. Colonies 1, 2, and 3 reduce the sugar D-mannitol and D-mannose. Further, colonies 1, 2, and, 4 reduce the sugars D-maltose and sucrose (except colony 2). Also, colonies 1 and 3 reduce D-trehalose (Table 5). The biochemical properties of bacterial colonies from threadfin bream were also detailed below as represented in Table 6 and Table 7. The enzyme tests of isolates of thread fin bream were observed as colonies1, 2, 3, and 4 produced enzymes such as tyrosine arylamidase and urease. Colonies1, 2, and 3 produced enzymes such as gamma-glutamyl transferase, alpha-glucosidase, phosphatase, and coumarate, which also obtained positive results for the Ellman and H_2S production test. Further, colony 3 showed positive results in L-proline arylamidase, citrate, L-malate assimilation, and O/129 resistance. Further, the colony 2 produced beta-glucosidase. Colony 4 was totally different and obtained varied test results from other colonies, as colony 4 showed the positive result for the enzyme such as L-pyrrolydonyl-arylamidase, L-aspartate arylamidase, leucine arylamidase, also a positive reaction to novobiocin resistance and optochin resistance test (Table 6). The carbohydrate fermentation tests of bacterial isolates from threadfin bream showed that colonies 1, 2, and 3 reduce the sugar D-maltose. Further, colony 4 reduces 4 sugars namely D-mannose, cyclodextrin, D-ribose, and salicin. Also, colony 3 alone reduces the sugar D-trehalose (Table 7). From, the above detailed biochemical results, the 4 bacterial colonies of Narrow-barred Spanish mackerel and the 4 colonies of Japanese thread fin bream were identified as *Aeromonas sobria, Morganella morganii, Hafnia alvei, Yersinia* sp, *Proteus* sp, *Proteus vulgaris, Proteus mirabilis* and *Vagococcus fluvialis* respectively (Table 8).

The fish samples such as mackerel and threadfin bream purchased from retail fish markets were subjected to microbial studies by conventional identification methods. In this study, it is confirmed that both scombroid and non-scombroid fishes are susceptible to histamine-producing bacteria. The average of total bacterial count and total histamine-producing bacteria were calculated (CFU/ml), and results show that the histamine-producing bacteria is1-2 log lowers than the total bacteria. The prevalence of histamine-producing bacteria (HPB) in both fish samples such as mackerel and threadfin bream gives a relatively same range of bacterial populations and its effects. To ensure the safety of fish and fish products, interventions are proposed throughout the production and supply chain. Prebiotics, symbiotics, probiotics, plant-derived antimicrobials, and vaccines are utilized to improve the resistance of fish against infections (Sheng and Wang 2021).

Table1.Total bacterial count and histamine producing bacterial count as CFU in Niven agar from Narrow-barred Spanish mackerel, *Scombromorus commerson* and Japanese Threadfin bream, *Nemipterus japonicus*.

Samples	Total bacterial count (CFU/ml)	Histamine-producing bacterial count(CFU/ml)
	10^{-5}	10^{-5}
Narrow barred-Spanish Mackerel	225×10^{-5}	48×10^{-5}
Japanese Thread fin Bream	180×10^{-5}	28×10^{-5}

Table 2. Primary tests for identification of bacterial colonies isolated from Narrow-barred Spanish mackerel, *Scomberomorus commerson*.

Bacterial colony	Motility	Gram staining	Endospore staining
Colony1	Motile	Gram negative	Vegetative cell
Colony2	Motile	Gram negative	Vegetative cell
Colony3	Motile	Gram negative	Vegetative cell
Colony4	Motile	Gram negative	Vegetative cell

Table 3. Primary tests for identification of bacterial colonies isolated from Japanese threadfin bream, *Nemipterus japonicus*.

Bacterial colony	Motility	Gram staining	Endospore staining
Colony1	Motile	Gram negative	Vegetative cell
Colony2	Motile	Gram negative	Vegetative cell
Colony3	Motile	Gram negative	Vegetative cell
Colony4	Motile	Gram positive	Vegetative cell

Table 4. Biochemical tests for enzyme production by bacterial colonies isolated from Narrow-barred Spanish mackerel, *Scomberomorus commerson*.

Biochemical tests	Narrow- barred- Spanish mackerel			
	Colony 1	Colony 2	Colony 3	Colony 4
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 alkalinisation	-	+	+	-
Alpha-Glucosidase	-	-	-	-
Succinate alkalinization	-	-	+	-
Beta-N-Acetyl-Galactosaminidase	-	-	-	-

Alpha-Galactosidase	-	-	-	-
Phosphatase	-	+	+	+
Glycinearylamidase	-	-	-	-
Ornithine decarboxylase	-	+	+	-
Lysine decarboxylase	-	-	+	-
Coumarate	+	-	-	+
Beta glucoronidase	-	-	-	-
Glu-Gly-Arg-Arylamidase	+	-	-	-
L-Lactate assimilation	-	-	+	-
H_2S production	-	-	-	+
L-Histidine assimilation	-	-	-	-
O/129 resistance	+	+	+	-
L-Malate assimilation	-	-	-	-
Ellman	+	+	+	+

Table 5. Biochemical tests for carbohydrate fermentation by bacterial colonies isolated from Narrow-barred Spanish mackerel, *Scomberomorus commerson.*

Biochemical tests	Narrow barred- Spanish mackerel			
	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	+	-	-	+
D-Tagatose	-	-	-	-
D-Trehalose	+	-	+	-

Table 6. Biochemical tests for enzyme production by bacterial colonies isolated from Japanese thread fin bream, *Nemipterus japonicus*.

Biochemical tests	Japanese thread fin bream			
	Colony 1	Colony 2	Colony 3	Colony 4
Ala-Phe-Pro-Arylamidase	-	-	-	-
L-Pyrrolydonyl-Arylamidase	-	-	-	+
Beta-Galactosidase	-	-	-	-
H_2S production	+	+	+	±
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 alkalinisation	+	-	+	-

Alpha-Glucosidase	+	+	+	-
Succinate alkalinization	+	-	+	±
Beta-N-Acetyl-Galactosaminidase	-	-	-	±
Alpha-Galactosidase	-	-	-	-
Phosphatase	+	+	+	-
Glycinearylamidase	-	-	-	±
Ornithine decarboxylase	-	-	-	±
Lysine decarboxylase	-	-	-	±
L-Histidine assimilation	-	-	-	±
Coumarate	+	+	+	±
Betaglucoronidase	-	-	-	-
O/129 resistance	-	-	+	-
Glu-Gly-Arg-Arylamidase	-	-	-	±
L-Malate assimilation	-	-	+	±
Ellman	+	+	+	±
L-Lactate assimilation	-	-	-	±
Phosphotidylinositolphospholipasec	±	±	±	-
Alpha-glucosidase	±	±	±	-
Arginine di hydrolase	±	±	±	-
L-aspartate arylamidase	±	±	±	+
Beta galactopyranosidase	±	±	±	-
Alpha-mannosidase	±	±	±	-
Leucine arylamidase	±	±	±	+
Betaglucuronidase	±	±	±	-
Alaninearylamidase	±	±	±	-
Polymixinbresistance	±	±	±	-
N-acetyl-D-glucosamine	±	±	±	-
Bacitracin resistance	±	±	±	-
Novobiocin resistance	±	±	±	+
Growth in 6.5% NaCl	±	±	±	-
Methyl-B-D-glucopyranoside	±	±	±	-
Argininedihydrolase 2	±	±	±	-
Optochinresistance	±	±	±	+

Table 7. Biochemical tests for carbohydrate fermentation by bacterial colonies isolated from Japanese thread fin bream, *Nemipterus japonicus*.

Biochemical tests	Japanese thread fin bream			
	Colony1	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	+	+	+	-
D-Tagatose	-	-	-	±

D-trehalose	-	-	+	-
D-amygdalin	±	±	±	-
D-xylose	±	±	±	-
Cyclodextrin	±	±	±	+
D-galactose	±	±	±	-
D-ribose	±	±	±	+
Lactose	±	±	±	-
Pullulan	±	±	±	-
D-raffinose	±	±	±	-
Salicin	±	±	±	+

Table 8. Identification of Histamine Producing Bacteria by using VITEK, a biochemical test analyzer.

Scomberomorus commerson	
Colony 1	*Aeromonas sobria*
Colony 2	*Morganella morganii*
Colony 3	*Hafnia alvei*
Colony 4	*Yersinia* sp
Colony 1	*Nemipterus japonicus* *Proteus* sp
Colony 2	*Proteus vulgaris*
Colony 3	*Proteus mirabilis*
Colony 4	*Vagococcus fluvialis*

Shalaby (1996) reported the presence of histidine decarboxylase in some certain *Entero bacteriaceae*, Clostridium, and *Lacto bacillus* species. Histamine-producing bacteria were reported in many other studies as *Morganella morganii* by Kimata (1961) and Arnold and Brown in 1978. Lerke *et al.*(1978) and Taylor *et al.*(1979) found *Klebsiella pneumoniae* as histamine-producing bacteria in Tuna fish likewise *Hafnia alvei* found by Ferencik (1970), *Proteus* sp., *Clostridium perfringens*, *Enterobacteraerogenes*, *Vibrio alginolyticus* founded in Skipjack tune by (Arnold *et al*. 1980), (Yoshinaga *et.al.* 1982) and Frank *et al*. 1985 discovered in Mahi-mahi species, *Bacillus* sp (Rodriguez-Jerez *et al*. 1994), histamine forming bacteria have been identified inhalotolerant *Staphylo coccus*, Vibrio, and Pseudomonas (Tsunami and Echigo, 1991, 1992). Psychrophilic and mesophilic halophilic histamine-forming bacteria have been isolated from marine fish (Okuzumi *et al*.1994), (Yogushi *et al*.1990) and (Tsunami and Echigo, 1992). In this study, of the total 8 strains of mackerel and thread fin bream, 5 strains belong to the family *Enterobacteriaceae* such as Hafnia alvei, *Yersinia sp*, Proteus *sp*, *Proteus vulgaris, Proteus mirabilis,* and others belong to *Aeromona daceae, Morganell aceae, Entero coccaceae* such as *Aeromonas sobria, Morganella morganii, Vagococcus fluvialis* respectively. One study reported that some of the gram - negative bacteria like Enterobacter sp., *Enterobacter cloacae, Erwinia persicina, Hafnia paralvei*, and *Klebsiella aerogenes, Serratia liquefaciens, and Shigella flexneri* were isolated from Salted-Boiled Longtail (Thunnus *sp*.) and Eastern Little Tuna (Euthynnus *sp.*) by Novalia Rachmawati in 2023. Marwa AE Refai *et al.* (2020) studied the most frequently isolated species were Klebsiella (33.3%) followed by staphylococci (24.7%), Salmonella (22.7%), *E. coli* (18.7%), Pseudomonas (18%), Proteus (16.7%) and Vibrio (6.7%) species. The colonies identified were similar to some of the strains discussed in the previous studies.

The HPB is found in a portion of the fish gut and gill microbiome; once the fish dies, the histamine-producing bacteria HPB invade the muscles and begin the transformation of histidine to histamine where the dead fish exposure to a temperature less than 4^{o}C for an extended period of time increases the activity of the HPB and fastens histamine accumulation while keeping the fish chilled immediately after catching prevents this process (Sabry *et al*., 2019).According to Bedane (2022), fish transportation and storage practices are not in line with the required standards. All food establishments did not have refrigerated vehicles for transportation, and only 30% of the retailers shipped fresh filleted fish using vehicles equipped with cold chain facilities. Previous studies on histamine-producing bacteria had reported that usually, dark muscle fishes produce high amounts of histamine-producing bacteria, so we chose to work on two white muscle fishes to examine whether the histamine-producing bacteria is present or not also to determine the rate of histamine-producing bacteria in the white muscle fishes. As a result, histamine-producing bacteria have been isolated from both white muscle fishes such as Narrow-barred Spanish mackerel and Threadfin bream. The histamine-producing

bacteria which were commonly present in dark muscle fishes at high rates such as *Morganella morganii*, *Hafnia alvei*, and Proteus species were also isolated from the chosen white muscle fishes. Factors affecting the growth of histamine-producing bacteria include the type and size of fish handling techniques and cooling methods (FDA, 2001). The samples collected were kept open in the market without ice preservation and proper maintenance which gives the opportunity for histamine-producing bacteria to proliferate.

CONCLUSION

The study revealed the presence of histamine-producing bacteria in both fish samples such as mackerel and threadfin bream purchased from Ukkadam fish market in Coimbatore. This considerable incidence of histamine-producing bacteria which on proliferation under the right circumstances may develop toxic histamine accumulation in the flesh of fishes. Also, the presence of histamine-producing bacteria indicates the improper maintenance of fish conditions in the retail fish markets in Coimbatore. Through this study, we concluded that improvement of hygienic practices in in-store retail markets and emerging control measures for delaying the formation of biogenic amine may reduce the health risk of food poisoning. To overcome the histamine poisoning in edible fishes, the fishes can be stored at 4^{o}C or below, by inhibiting the growth of bacteria.

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