Histidine decarboxylase activity of enteric bacteria in fish/shellfish from retail markets of Cochin, India

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Abstract

Histidine decarboxylase activity of Enterobacteriaceae isolates from 104 samples of fish and shellfish samples collected from retail markets of Cochin was studied. The total Enterobacteriaceae count varied from 2.5 to 6.5 log cfu g^{-1} among the different fish species. Generic level characterization of 248 enteric bacteria showed that Enterobacter Spp., *and* Escherichia Spp. *were the dominant groups. All the isolates were checked for their ability to decarboxylase histidine and the isolates showing positive reaction from different sample sources were reported. Effect of five different incubation temperatures on the decarboxylase activity of these isolates was also* evaluated.

Keywords: *Enterobacteriaceae*, Seafood, *Histidine* decarboxylase activity, *Temperature effect*

1. Introduction

Histamine Fish Poisoning (HFP) or Scombroid fish poisoning is a significant public health and safety concern (Lehane and Olley, 2000). Scombroid fish belonging to the families Scom-bridae (*e.g.*, tuna and mackerel) and Scom-beresocidae (*e.g.*, saury) are most commonly associ-ated with HFP, but non-scombroid species (*e.g.*, mahi-mahi, sardines, pilchards, anchovies, herring, marlin and blueûsh) can also be involved. Histamine production in fish is related to the histidine content of the fish, the presence of bacterial histidine decarboxylase (HD), and environmental conditions (Lehane and Olley, 2000). Histamine is formed post mortem in fish by proliferation of bacteria synthesizing histidine decarboxylase to convert histidine to histamine (Taylor *et al.*, 1978).

Histamine is reportedly produced by a wide range of microorganisms, the vast majority of which are gram-negative rods of the Enterobacteriaceae family (Taylor *et al.*, 1978, Lehane and Olley, 2000, Lopez-Sabater *et al.*, 1994). It includes *Morganella morganii*, certain strains of *Klebsiella pneumoniae* and few starins of *Hafnia alvei* that are often incriminated (toxicologically significant amounts of

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histamine) in scombroid poisoning (Lehane and Olley, 2000, Lopez-Sabater *et al.*, 1996, Niven *et al.*, 1981). Other species *Citrobacter freundii, Enetrobacter clocae, E. aerogenes, Proteus vulgaris, P. mirabilis, Serratia fonticola, S. liquefaciens, Escherichia coli, Salmonella spp. etc* are also involved. (Niven *et al.*, 1981, Lopez-Sabater, *et al.*, 1996, Lopez-Sabater *et al.*, 1994). In addition to enteric bacteria, members of the genera *Vibrio, Photobacterium, Clostridium, Aeromonas, Plesiomonas, Pseudomonas, Acinetobacter* and N-group bacteria are also reported as histamine producers. (Niven *et al.*, 1981, Taylor et al., 1978, Okuzumi *et al.*, 1984, Lehane and Olley, 2000). Certain strains of *Lactobacillus* that are also prolific histamine producers are proba-bly of importance only in fermented fish (Taylor *et al.*, 1978).

Several factors affect bacterial growth and histidine decarboxylase activity. These include incubation temperature (Behling and Taylor, 1982), pH of medium (Okuzumi *et al.*, 1984), the concentration of histidine and carbohydrates in the medium, oxygen tension, vitamins and co enzymes (Lehane and Olley, 2000).

The present study was carried out to determine the histidine decarboxylase activity of Enterobacteriaceae and to evaluate the effect of five different incubation temperatures on the isolates from different fish and shellfish samples collected from retail markets of Cochin

2. Materials and methods

Fish samples from five selected markets in and around Cochin, India were included in the study. A total of 104 samples comprising Indian Oil Sardine - *Sardinella longiceps* (17), Indian Mackerel - *Rastrelliger kanagurta* (17), Thread fin bream - *Nemipterus japonicus* (17 nos), Prawn - *Metapenaeus dobsoni (17), Pearlspot - Etroplus suratensis* (17), Black Clam - *Villorita cyprinoides* (12) and boiled clam meat - *Villorita cyprinoides (7) were analysed during the study.*

Quantitative estimation of total Enterobacteriaceae was carried out by direct plating on Violet Red Bile Glucose Agar (VRBGA, Oxoid, UK) with incubation at 37 °C for 18-24 h. For identification 2-5 well separated typical colonies were selected using Harrison's disc method. These cultures were purified and stored for further study in nutrient agar slants. Altogether 248 pure culture isolates were obtained and identified up to the genus level. Source of these isolates were given in Table 1. For identification a scheme from web http//www.vet.uga.edu/WEBFILES/ in consultation with Edwards and Ewings (1978) was used. About 5 % of the isolates were crosschecked with Analytical Profile Index 20 E (API 20 E, bioMerieux) for confirmation.

Table 1. Distribution of HDB in various fish samples and Markets

Fish Species	Market I	Market II	Market III	Market IV	Market V
Indian oi l sardine (44)	0 (8)	0 (10)	0 (10)	0 (8)	0 (8)
Indian Mackerel (52)	0 (10)	8 (12)	0 (10)	0 (10)	0 (10)
Prawn (52)	0 (10)	2 (12)	0 (10)	0 (10)	0 (10)
Pearl spot (40)	0 (8)	2 (8)	0 (8)	0 (8)	2 (8)
Thread fin bream (30)	0 (6)	0 (6)	0 (6)	0 (6)	0 (6)
Fresh Clam (20)	-	-	-	0 (10)	0 (10)
Boiled Clam (10)	-	-	-	0 (5)	0 (5)

Numbers in parenthesis indicate total isolates studied

All the 248 isolates were tested for the histidine decarboxylase activity in Niven's medium comprising 0.5 % tryptone, 0.5 % yeast extract, 0.5 % NaCl, 0.1 %, $CaCO_3$, 0.006 % bromocresol purple, and 2.7 % histidine-2HCL, pH 5.3 (Niven *et al.*, 1981). Those isolates showing positive reaction in Niven's medium were tested for its potential to decarboxylase histidine at different temperatures *viz.* 6 °C, 13.5 °C, RT (30 ± 2 °C), 37 °C and 42 °C.

3. Results and discussion

3.1 Quantitative and Qualitative study of Enterobacteriaceae in fish/shellfish

The total Enterobacteriaceae count among the different fish/ shellfish samples varied from 2.5 to 6.5 log cfu g^{-1}. The percentage of total Enterobacteriaceae in the total aerobic bacteria for different samples from different markets is presented in Table 2. The values range from as low as 0.075 % to the highest value of 11.59 %. All the samples in the study carried an enteric bacterial count less 3 % of total aerobic bacteria. Exceptions noted were for mackerel samples from Kadavanthara and Etroplus from Kaloor market.

At generic level identification of 248 enteric isolates from different fish and shellfish, eleven genera could be detected. *Enterobacter spp.*, *Escherichia spp.*, and *Edwardsiella spp.*, were the dominant genera detected in all seafood samples, but their relative proportion varied with samples and the data were comparable with earlier studies (Souter *et al.*, 1976; Sangjindavong and Cjerde, 1977). Other groups, in the order of dominance were *Citrobacter spp.*, *Arizona spp.*, *Hafnia spp.* and *Shigella spp.* The genus *Klebsiella* and *Proteus* could be obtained only from *Nemipterus* and Clam samples. Presence of genera *Shigella* spp. and *Arizona spp* is not reported previously (Souter *et al.*, 1976; Sangjindavong and Cjerde, 1977). At the same time the present study failed to recover *Salmonella spp.* and *Serratia* spp. which was recovered

by Souter *et al.*, (1976). *Aeromonas* a non Enterobacteriaceae was also observed in the study.

Table 2. Percentage distribution of Total Enterobacteriaceae in the total plate count

Samples / Markets	Sardine	Mackerel	Prawn	Etroplus	Nemipterus	Boiled Clam	Fresh Clam
Market I	1.9	1.11	0.075	0.16	0.47	N.A	N.A
Market II	1.66	0.5	0.35	0.195	N.A	N.A	N.A
Market III	0.21	11.59	0.7	0.8	0.78	N.A	N.A
Market IV	0.3	0.73	0.3	10.6	N.A	47.3	71
Market V	3.198	0.79	0.25	2.32	N.A	0.305	0.12

N.A-Not Analysed

3.2 Histidine decarboxylation by the members of the family Enterobacteriaceae

The results of the histidine decarboxylase activity of 248 enteric isolates from fish/shellfish samples were shown in Table 3. It was observed that only 5.64 % of the total isolates decarboxylated histidine. This is in contrary to the study of Lopez-Sabater *et al.*, (1993) where in the authors reported that 87 % of histidine decarboxylating bacteria belonged to the gram negative Enterobacteriaceae. Among the fourteen isolates which were positive for the HD enzyme activity, ten belonged to the genera *Citrobactor spp*, two isolate belonged to *Edwardsiella spp* and the other two genera is *Providencia spp*. No other genera, namely *Klebsiella spp* (12 nos), *Escherichia spp* (55 nos), *Proteus spp* (11 nos.) and *Enterobactor spp* (83 nos) obtained from fish/shellfish showed this activity.

Table 3. Histidine decarboxylase activity by members of the family Enterobacteriaceae

acterial genera	Aeromonas	Arizona	Citrobacter	Enterobacter	Escherichia	Edwardsiella	Hafnia	Klebsiella	Proteus	Providencia	Shigella	Untypable	Total isolates
No of isolates tested	6	2	38	83	55	19	2	12	11	12	2	6	248
HDpositives	0	0	10	0	0	2	0	0	0	2	0	0	14
			(71.4%)			(14.3)				(14.3)			
Percentage	0	0	26.31	0	0	10.5	0	0	0	16.6	0	0	5.64

HD-Histidine decarboxylase

Subburaj *et al.*, (1984) reported that histidine decarboxylase activity and histamine formation vary with species of bacteria as there was no direct correlation between HDB count and histamine level in multiple fish samples. Lopez-sabater *et al.*, (1993) described *Morganella morganii, Klebsiella oxytoca, K. pneumoniae* as prolific histamine formers (>1,000 ppm), *Enterobacter cloacae, E. aerogenes* as frequently isolated one from fish (500-1,000 ppm) and third category of less potent ones *Citrobacter freundii, Proteus mirabilis, Enterobacter agglomerans, Proteus vulgaris* and *Serratia liquefaciens* (<250 ppm). But in the present study *M. morganii* was not detected among the enteric bacterial population. No other genera described as prolific histamine producing strain was found to be positive for the enzyme activity. Even those described as frequently isolated ones also failed to decarboxylase histidine, only organism described under the last category, that is slow histamine producers (*Citrobacter spp.* and *Providentia Spp. Edwardsiella spp.*) were shown positive reaction for HD in the present study.

Out of the 38 isolates of *Citrobacter* only 10 (26.31 %) strains have shown the HD enzyme activity. Many have recovered *Citrobacter* spp especially *Citrobacter freundii* as HDB (Taylor *et al.*, 1979, Niven *et al.*, 1981, Lopez-Sabater *et al.*, 1994). However, the percentage contribution of *Citrobacter* among the HDB isolated from different marine fishes were always minimum (Lopez-Sabater, *et al.*, 1996; Gopakumar *et al.*, 1988). Gopakumar *et al.*, (1988) reported the recovery of *Escherichia coli* as HDB. All the 55 isolates of *Escherichia spp* in this study, failed to decarboxylase histidine.

Among the ten *Klebsiella spp.* and one *Hafnia spp.* tested, all of them were negative to the test. Taylor *et al.*, (1979) isolated a high-histamine-producing strain of *K. pneumoniae* from a sample of tuna sashimi impli-cated in an outbreak of HFP and this strain was identified as *Klebsiella planticola* ATCC 43,176. This strain together with *K.ornitholytica* and *K.terrigena* were classified under a new genus *Raoultella* (Guirard and Snell, 1987). Due to misidentification of *R. planticola* as either *K. pneumoniae* or *K.oxytoca, K. pneumoniae* was often reported as histamine producer. In a recent study involving a collection of 61 strains of *K. pneumoniae* and 18 strains of *K. oxytoca* from fish produced no histamine and only those identified as *R. ornithinolytica* (5 strains), *R. planticola* were isolated as new HPB strains (Kanki *et al.*, 2002). As the isolates in this study were characterized only up to genus level, complete identification is not possible.

Among the 12 *Providencia spp*, only two isolate has shown the HD activity. In an earlier study *Providencia* contributed 8 % of the HDB in mackerel sample (Subburaj *et al.*, 1984). In the case of *Proteus*, all the 6 isolates were not able to decarboxylase histidine even though they

are considered as active HDB (Lopez-Sabater, *et al.*, 1996; Gopakumar *et al.*, 1988).

A common feature of HDB is that multiple strains of the same species show variations in histamine formation. (Leung, 1987; Taylor *et al.*, 1978; Taylor *et al.*, 1979). In attempting to explain this aspect, Leung (1987) postulated that HD may be controlled by a plasmid and that the plasmid may be transferred from one strain to another, species to species or genus to genus.

Further, the source of the isolates was traced (Table 2). Out of 14 positives 12 isolates were from market II and two from market V. Regarding the sample sources, out of 14 positive isolates for histamine production, 8 were from Indian mackerel (6 *Citrobactor spp.* and 2 *Providencia spp*). Two of *Citrobactor spp.* and 2 of *Edwardsiella spp.* from Etroplus showed HD activity. The last two isolates (*Citrobacter spp.)* were from prawn samples. It is proved from the above observation that apart from mackerel, two other categories of fish, Etroplus and Prawn, can also harbour the histidine decarboxylating bacteria.

3.3. Effect of Incubation Temperature on Histidine decarboxylase activity

In addition to other factor, temperature of storage plays a paramount role in the HFP. In this study all the histidine decarboxylase positive isolates of enteric origin obtained from fish/shellfish were tested for its potential to decarboxlase histidine at different temperatures viz. 6 °C, 13.5 °C, room temperature (30 ± 2 °C), 37 °C, 42 °C. All the 10 *Citrobacter spp* were able to decarboxylate histidine at RT or 37 °C (Table 4). This observation agrees with previous work that the lower limits for the production of toxicologically significant levels of histamine in tuna fish infusion broth for *Citrobacter freundii* is 30 °C (Behling and Taylor, 1982). For other common HDB the limits reported were 7 °C for *K. pneumoniae*, 15 °C for two *M. morganii* strains (the maximal HD activity at 37 °C), and 30 °C for *H. alvei* and *E. coli.*

Table 4. Effect of Incubation temperature on histidine decarboxylase activity by the members of Enterobacteriaceae

Incubation Temperature						
Genera	**No of isolates**	6.5 °C	13.5 °C	RT (30±2 °C)	37 °C	42 °C
Citrobacter	10	-	-	+	+	-
Providencia	2	-	-	+	+	-
Edwardsiella	2	-	-	+	+	-

There are studies on the effect of storage temperature on histamine formation in fish but their results are quite often ambiguous as different authors reported different temperature ranges of HD activity for same species of bacteria (Lopez-Sabater *et al.*, 1994, Lehane and Olley, 2000, Lopez-Sabater *et al.*, 1996). Doe *et al.*, (1998) in trying to classify HDB based on temperature showed that HDB has no clear cut temperature range and it can be found across most of the temperature–growth spectrum from 0 °C to 65 °C. The author has described that all the Enterobacteriaceae fall under the group C *growth-temperature spectrum which is nothing but the tropical* ambient temperature. In this study also all the 14 isolates were found to have the histidine decarboxylase ability at tropical ambient temperature (30 ± 2 °C) and 37 °C.

At 6 °C, and 13.5 °C none of the isolate tested was able to decarboxylase histidine. Lehane and Olley (2000) pointed out that certain microorganisms have the low-temperature HD enzyme activity. However, they can be significant in the point of view of histamine poisoning only when sufficient bacterial numbers have been reached before the product was stored at low temperature.

4. Conclusions

The scombriod toxicity, which is due to the decarboxylasion of histidine to histamine is common in fishes and members of Enterobacteriaceae, is reported to elicit decarboxylase property. Among the 11 genera of Enterobacteriaceae isolated, a limited number of starins identified as *Citrobacter, Edwardsiella* and *Providencia* were able to decarboxylase histidine. This HD activity of these isolates were observed at room temperature (30±2 °C) and 37 °C only.

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