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Histamine and Histamine Producing Bacteria in Fish from Sri Lanka

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ABSTRACT. *Histamine is produced in fish by spoilage bacteria of the family Enterobacteriaceae. The amino acid, histidine, in fish is decarboxylated to histamine by some of the bacteria.*

Presence of histamine was determined in fresh fish, and dried fish of 21 types. Histamine was extracted by blending 5 g of fish with 35 ml methanol and maintaining the blend at 60°C for 15 min. The filtrate was examined on silica gel G60 by Thin Layer Chromatography.

Members of the Enterobacteriaceae were isolated from fish using Violet-red-bile-glucose-agar. Histamine producing bacteria among these isolates were selected using Niven's medium (Niven, Jeffrey and Corlett, 1981). Histidine decarboxylating activity was examined by inoculating suspected cultures into sterile Niven's broth and a histamine-free 40% aqueous fish blend of Herring, Skipjack or Tuna. The inoculated media were incubated at 37°C for 7 days followed by determination of histamine. A colour change of Niven's medium to purple was indicative of histamine production. A bacterial culture isolated from a sample of Herring gave positive reactions in both Niven's broth and a fish blend.

Fresh Tuna, Skipjack, Herring, Shark, and dried Skipjack and Sprats contained histamine in 12.5, 13.0, 15.7, 13.0, 60.0 and 64.0% of samples tested, respectively. Of 156 samples tested 48 were positive for Enterobacteriaceae. Of the 48 cultures, 25 were histamine producing bacteria. The cultures originated from fresh Indian Mackerel, Tuna, Skipjack, Herring, Shark, Squid and Trevally.

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INTRODUCTION

Histamine is produced in the scombroid fish (family Scombridae and Scomberosocidae) from the amino acid, histidine. The types of fish most commonly reported to undergo histamine formation include varieties of Tuna, Skipjack, Bonito and Mackerel (Frank and Yoshinaga, 1984). Fresh scombroid fish have virtually no histamine in their muscle, but, after storage at ambient temperatures the fish acquire substantial amounts of histamine.

Histamine is produced in fish by several bacteria of the family Enterobacteriaceae. The most frequently implicated organisms are *Klebsiella pnuemoniae*, *Morganella morganii* and *Hafnia alvei* (Chen *et. al.*, 1989). They decarboxylate the amino acid, histidine in fish into histamine. Histamine causes adverse reactions in certain allergic individuals on consumption of fish. Redness in eyes, tingling and burning sensations around mouth, gastro-intestinal complaints and rashes with itching are common symptoms of histamine poisoning (Kottegoda, 1984). The presence of histamine in Skipjack, and the allergic reactions caused by it in individuals are already reported in a study in Sri Lanka (Uragoda and Kottegoda, 1977). Production of histamine in fish, after catching, may occur at sea, before and after landing, during transport from landing sites to markets and at the markets. Production of histamine generally occurs near the skin and in the intestinal tract, as the bacteria are present in larger numbers in these areas.

A survey of histamine in fresh and dried-fish was carried out, and the histamine producing bacteria (HPB) were isolated. The ability of the isolated bacteria to produce histamine in laboratory cultures was examined at ambient temperature (25 $\pm$2)°C and 37°C.

MATERIALS AND METHODS

Samples

Fish and dried-fish were collected at random from retail shops in Peradeniya and Kandy. One hundred and fifty six (156) samples belonging to 21 types of fresh fish and dried-fish were tested. Fresh fish, Skipjack (*Katsuwonus pelamis*), Tuna (*Thunnus albacares*), Sprats (*Anchoviella commersonii*), Barracuda (*Sphyraena jello*), Devil ray (*Mobla diabolus*), Flying fish (*Exocoetus volitans*), Herring (*Ambligaster sirm*), Indian

Mackerel (*Rastrelliger kanagurth*), Sailfish (*Histiophorus gladius*), Sardine (*Sardinella longiceps*), Shark (*Carchahinus* sp.), Silverline Half Beak (*Hyporhampus jussimieri*), Spanish Mackerel (*Scomberomorus commersoni*), Trevally (*Caranx stellatus*), Squid (*Sepia* sp.), Crab (*Cancer magister*), Tilapiya (*Tilapiya* sp.), Mullet (*Mugil cephalus*), Prawn (*Penaeus indicus*) and the dried fish, Skipjack, Anchovies (*Thrissocles* sp.), Katta (*Chorinemus* sp.), Sprats, Herring, Spanish mackerel and Shark were analyzed for histamine and histamine producing bacteria.

Isolation of bacteria

As soon as the samples were received in the laboratory, a small portion with the skin was aseptically transferred into sterile 1% peptone water. A loopful of this solution was inoculated into Violet-red-bile-glucose-agar and incubated at 37°C for 5 days. Positive colonies giving pink colour were transferred into Niven's medium and incubated at ambient temperature and 37°C for 2 days. Positive cultures giving a purple colour reaction or a purple halo were maintained on agar slants and periodically subcultured (Chen *et. al.*, 1989; Ababouch *et. al.*, 1991).

Histamine production

Histidine decarboxylating activity of the isolated pure bacterial cultures was examined by inoculating into Niven's broth and sterile 40% fish blends. The fish blends were prepared by blending histamine free fish, in 1% peptone water, in a Waring blender, at low speed, for 2 min. The inoculated broths and blends were incubated at ambient temperature (25±2)°C and 37°C for 7 days followed by examination for histamine production (Niven *et. al.*, 1981).

Detection of histamine

Histamine produced in fish was detected by blending 5 g of sample for 2 min with 35 ml of methanol at low speed in a Waring blender, followed by maintaining the blend in a water bath at 60°C for 15 min to enhance extraction. The extract was allowed to cool to room temperature, in a graduated conical flask, made to 50 ml with methanol and filtered. The filtrate was used for spotting on thin layer chromatographic plates pre-coated

with silica gel G60 and activated at 105°C for half an hour. Acetone: conc. ammonia (95:5) in an equilibrated tank was used for developing the TLC plates, followed by visualization with ninhydrin spray and comparison with authentic samples of histamine (Lieber and Taylor, 1978; Baranowski, 1985).

RESULTS AND DISCUSSION

Histamine in market fish

Of the 156 samples of fresh fish and dried-fish belonging to 21 types, fresh Tuna, Skipjack, Herring and Shark contained histamine in 12.5%, 13.0%, 15.7%, and 13.0% of the samples tested, respectively. The dried-Skipjack and dried-Sprats contained histamine in 60.0% and 64.0% of the samples tested, respectively (Table 1). Although the tuna type fish are well established histamine producers, the authors have not come across any reports of histamine in dried-Sprats. Except for dried-Sprats the other types of fish have been reported to contain high histamine contents by Lieber and Taylor (1978) and Baranowski (1985).

It was also noted that the incidence of histamine in dried-Skipjack and dried-Sprats was higher than in fresh samples of the same fish. This probably may be due to greater opportunity for the histamine producing bacteria to grow during the drying of fish.

Histidine decarboxylating bacteria in fish

Of the 156 samples of fish tested, 48 gave positive reactions for Enterobacteriaceae, of which, 25 cultures gave positive reactions as histamine producing bacteria (HPB).

A higher number of Enterobacteriaceae cultures was isolated from the fresh fish than from dried-fish, in contrast to a higher incidence of histamine in dried-fish. Enterobacteriaceae were isolated from 43 out of 119 fresh fish samples, whereas only 5 of 37 dried-fish samples contained the same (Table 2). A limitation in this study was that the fresh fish collected may have been stored in ice during transport to Kandy, resulting in a loss of viability of some of the histamine producing bacteria. Niven, Jeffrey and Corlett (1981) have stated that bacteria responsible for histamine production were

difficult to detect, after freezing of fish. The effect of drying on Enterobacteriaceae in fish is not known.

Table 1. Presence of histamine in fresh fish and dried-fish.

Type of samples	Number of samples tested	+ve for histamine	% of +ve samples
Fresh fish			
Tuna	16	2	12.5
Skipjack	15	2	13.0
Herring	19	3	15.7
Shark	15	2	13.0
Trevally	8	0	0.0
Sardine	5	0	0.0
Others	41	1	2.0
Dried fish			
Skipjack	5	3	60.0
Sprats	14	9	64.0
Herring	4	0	0.0
Others	14	3	21.0
TOTAL	156	25	16.0

Note. Other fish types not listed individually due to the small number of samples tested, include Barracuda, Indian Mackerel, Squid, Spanish Mackerel, Silverline half beak, Devil Ray, Sailfish, Prawn, Crab, Mullet, Flying fish, Anchovy, Katta and Tilapiya.

Histamine production in laboratory cultures

Histamine production was observed in Niven's broth and fish blend inoculated with a bacterial culture originating from a sample of Herring. Niven's broth also showed a colour change from yellow to purple.

Table 2. Isolation of Enterobacteriaceae and histamine producing bacteria (HPB) from fresh fish and dried-fish.

Type of sample	Number of samples		+ve for HPB
	tested	+ve for Enterobacteriaceae	
Fresh fish			
Tuna	16	7	5
Skipjack	15	7	4
Herring	19	7	3
Shark	15	7	4
Trevally	8	3	3
Sardine	5	1	0
Others	41	11	6
Dried fish			
Skipjack	5	1	0
Sprats	14	2	0
Herring	4	0	0
Others	14	2	0
TOTAL	156	48	25

Note: as in Table 1.

The histamine production appeared higher at 37°C than at ambient temperature (25±2)°C as observed by colour intensity on TLC. This was observed by lower colour intensity on TLC assay at (25±2)°C when compared with 37°C. The absence of colour change in Niven's broth at (25±2)°C when compared with a distinct colour change observed at 37°C also supported the above observation. This is in agreement with Frank, Yoshinaga and Wai-Kit Nip (1981), and Frank and Yoshinaga (1984) who reported that 37°C was distinctly superior to ambient temperature for the growth of bacteria and histidine decarboxylating activity. The available reports on effect of temperature on bacterial growth and histamine

production indicate that temperatures above freezing point of water support histamine production (Wei *et. al.*, 1990). However, prolonged storage for 42 days at -1.1°C was needed to produce 2 mg histamine per 100 g of Tuna (Frank and Yoshinaga, 1984).

CONCLUSIONS

Sprats, Skipjack, Tuna and Herring were found to contain histamine. Niven's medium could be successfully used to isolate HPB under tropical conditions. HPB showed a distinct preference for histamine production at 37°C compared to ambient temperature (25±2)°C.

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