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Histamine-Producing Bacteria in Decomposing Skipjack Tuna (*Katsuwonus pelamis*)†

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Spoilage in skipjack tuna (*Katsuwonus pelamis*) was studied under controlled conditions by incubating whole, fresh fish in seawater at 38°C, the optimum temperature for histamine formation. Bacterial isolates were obtained from the loin tissue of a decomposing tuna containing 134 mg of histamine per 100 g and a total anaerobic count of 3.5×10^5/g after incubation for 24 h. Over 92% of the 134 isolates obtained were facultatively or obligately anaerobic bacteria. Eighteen isolates produced histamine in culture media containing histidine, and these were identified as *Clostridium perfringens, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis*, and *Vibrio alginolyticus*. Histidine decarboxylase activity of several isolates was measured in a tuna broth medium and with resting cells suspended in a buffered histidine solution.

Fresh scombroid fish such as tuna, bonito, mackerel, etc., have virtually no histamine in their muscle (11–15), but after decomposition (spoilage), these fish contain substantial amounts of histamine. The histamine is produced by bacteria that decarboxylate histidine, an amino acid that is abundant in scombroid fish muscle (18, 23, 28). A large and diverse group of bacteria have been reported to be responsible for the histamine found in fish, dairy products, and even some vegetable products (1, 2, 10, 18, 19, 21, 24, 26, 27, 30, 31, 33, 34).

Large-scale commercial tuna fishing is done primarily by the purse seining method in which enormous nets are used to trap schools of fish. However, the netted fish cannot always be collected immediately but must remain in the ocean for a considerable time until they can be brought on board the fishing vessel and cooled before being frozen and stored. If these delays are prolonged, some postmortem decomposition can occur in the fish. Comparable delays also may happen when unusually large loads of fish must be cooled or when the equipment experiences mechanical failure.

In the present investigation, we studied decomposition in skipjack tuna under controlled conditions that simulated lengthy postmortem immersion of the fish in warm marine waters. Laboratory incubation studies were conducted in seawater at 38°C, the optimum temperature for histamine formation in skipjack tuna (12). Undesired spoilage changes were prevented by keeping the fish alive until shortly before initiating the experiments.

Most reports on the distribution of bacteria in marine fish consider the skin and gills as the major sources of spoilage organisms (26, 30, 34). Nevertheless, because the loins are the locus of histamine formation and microbial proliferation during tuna spoilage, we chose to isolate representative spoilage organisms from the muscle of a decomposed fish in this study. In addition, because the loin tissue is essentially anaerobic, we were interested in examining the effect of anaerobic conditions on histamine formation and the role of anaerobic bacteria in tuna spoilage. Although *Clostridium perfringens* is often included among the histidine-decarboxylating organisms (18, 24, 31, 34), no previous studies have dealt with histamine formation in scombroid fish under anaerobic growth conditions.

The present study shows that a variety of bacteria isolated from decomposed skipjack tuna, including *C. perfringens*, are capable of decarboxylating histidine. Our results suggest that histamine formed during decomposition of skipjack tuna could have been produced by several different organisms in the microflora, even under anaerobic conditions.

(A preliminary report of this work appeared previously [D. H. Yoshinaga and H. A. Frank, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, P25, p. 199]).

MATERIALS AND METHODS

Tuna. Locally caught skipjack tuna, each weighing about 2 kg, were kept alive for 12 to 18 h in storage tanks containing seawater and transported on ice to the laboratory shortly before experimentation (12).

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Incubation. Individual fish were placed in separate polyethylene bags containing 4 to 5 liters of filtered fresh seawater and incubated for the desired time at 38°C in a water bath.

Bacteriological enumeration. After incubation, the fish were eviscerated and decapitated, and each side was cut transversely into four or five sections. The sections were numbered consecutively, with no. 1 being the most anterior section and no. 5 the most posterior section (12). Microbial counts were made with the second section of the right side of each fish. This section was skinned and deboned aseptically, and the flesh was homogenized with 4 parts of sterile 0.1% peptone (BBL Microbiology Systems, Cockeysville, Md.) in a Waring blender. The homogenate was diluted in 0.1% peptone, inoculated on duplicate plates of Trypticase soy agar (BBL Microbiology Systems), and kept in an anaerobic incubator for 24 to 48 h at 38°C before colonies were counted.

Histidine content. Portions from different sections of a fresh tuna were freeze-dried and analyzed for histidine by the high-pressure liquid chromatographic procedure of Khayat et al. (A. Khayat, P. Redenz, L. Gorman, and D. Hopkin, Abstr. Annu. Meet. Inst. Food Technol. 1981, abstr. no. 267, p. 15).

Histamine content. The left side of the fish was steam heated for 15 min at 104°C in a home-style pressure cooker, and section no. 2 was removed for histamine analysis. This section was debrided, comminuted twice in a household meat grinder, and analyzed by the fluorometric histamine method used previously (3, 12).

Isolation and identification of tuna microflora. Spoilage microflora was isolated from section no. 2 of the right side of a tuna that contained 134 mg of histamine per 100 g after incubation for 24 h at 38°C. Procedures for preparation and plating of tuna samples were described above. Duplicate sets of Trypticase soy agar plates were inoculated and incubated at 38°C under aerobic and anaerobic conditions. Isolates of the spoilage bacteria were obtained from each set by subculturing all of the colonies on an aerobic and an anaerobic plate from identical dilutions of the tuna sample.

Aerobic isolates were gram-positive cocci and were identified by the catalase test and carbohydrate fermentations (5) and from colony morphology and various biochemical tests (32).

Facultatively anaerobic isolates were gram-negative rods that consisted of oxidase-negative *Enterobacteriaceae* and oxidase-positive *Vibrionaceae*. *Enterobacteriaceae* were identified by differential media and carbohydrate tests (8) from descriptions in *Bergey's Manual of Determinative Bacteriology, 8th ed.* (6). *Vibrionaceae* were identified by testing for sensitivity to vibriostatic agent 0/129 (BDH, Poole, England [British Drug House]), 2,4-diamino-6,7-diisopropylpteridine, and biochemical properties as described by Bain and Shewan (4) and *Bergey's Manual of Determinative Bacteriology, 8th ed.* (6).

Obligately anaerobic cultures were isolated and characterized by methods described in the Virginia Polytechnic Institute *Anaerobe Laboratory Manual, 4th ed.* (17) and identified by the computer-assisted method of Kelley and Kellogg (20).

Stock cultures of aerobic and facultatively anaerobic isolates were maintained on Trypticase soy agar slants and subcultured periodically to ensure viability. Obligately anaerobic isolates were maintained in cooked meat medium (Difco Laboratories, Detroit, Mich.) under a Vaspar anaerobic seal.

Screening for histidine decarboxylase-positive bacteria. Isolates were tested for histidine-decarboxylating activity in agar stab cultures of the differential medium of Niven et al. (25). A modification of Niven's medium was made by adjusting the pH to 6.5 to permit growth of acid-sensitive clostridia; this medium contained 0.5% tryptone (Difco), 0.5% yeast extract (Difco), 2% L-histidine (free base), 0.5% NaCl, 1.5% agar, and 0.02% cresol red. Histidine-decarboxylating activity of all isolates was tested with modified Niven's medium in (i) broth cultures containing inverted Durham tubes (6 by 50 mm) as carbon dioxide gas traps, (ii) agar surface streak cultures, and (iii) agar stab cultures (containing 0.1% glucose). A given strain was considered positive for histidine decarboxylase if gas was formed in the broth culture or if the indicator showed an increased pH in one or both of the agar media.

Histamine production by tuna isolates. Growth and histamine formation were measured in tuna fish infusion broth (TFIB) (26) prepared from fresh skipjack tuna. Duplicate sets of tubes containing 10 ml of TFIB were inoculated with 1 drop each from 24-h cultures in Trypticase soy broth (BBL Microbiology Systems) of 14 histidine decarboxylase-positive isolates and *Proteus morganii* no. 180, a histamine-forming strain obtained from D. A. Corlett, Jr., Del Monte Research Center, Walnut Creek, Calif. One set of TFIB tubes was incubated aerobically and the other set anaerobically for 48 h at 38°C. Viable counts were estimated in Trypticase soy agar plates, and the histamine content was measured in each tube.

Histamine formation by resting cell suspensions of some isolates was measured and compared with that observed with *P. morganii* no. 180 and with *Klebsiella pneumoniae* no. T2, a histidine decarboxylase-positive strain obtained from S. L. Taylor, Letterman Army Institute of Research, San Francisco, Calif. Cells were harvested from Trypticase soy broth after growing for 24 to 36 h at 38°C, washed several times by centrifugation, and suspended in sterile 0.2 M sodium phosphate buffer (pH 6.0) containing 0.1% histidine. After anaerobic incubation for 24 h at 38°C, the suspensions were filtered, and their histamine level was measured in the cell-free solution.

RESULTS

Histidine content of fresh tuna. Histidine is distributed uniformly at a concentration of about 578 mg/100 g throughout the fish. (The mean histidine contents [mg/100 g] for both sides of fish sections 1, 2, 3, and 4 were 611, 573, 565, and 564, respectively.)

Growth and histamine formation during tuna decomposition. Figure 1 shows the rate of bacterial growth and histamine formation in section no. 2 of skipjack tuna undergoing decomposition at 38°C. Growth was measured by the anaerobic bacterial count because over 92% of the isolates in decomposed tuna were obligately or facultatively anaerobic, and these included all of the

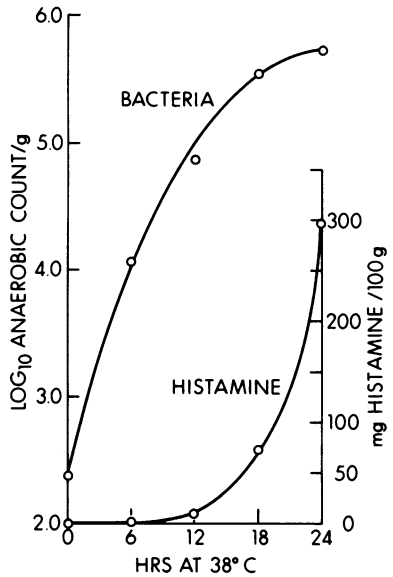

FIG. 1. Bacterial growth and histamine formation in skipjack tuna at 38°C. Fish were placed in plastic bags containing seawater, removed at the desired intervals during incubation, and analyzed for bacterial count and histamine content. The data shown were obtained from section no. 2 of each fish; anaerobic bacterial counts were made with the right side and histamine estimations with the left side of the fish. Anaerobic counts are the means calculated from two to four separate fish incubated at each of the time intervals shown. For details see the text.

isolates capable of producing histamine (Table 1). From an initial level of about 200/g, the anaerobic count rose to 3.5×10^5/g after 24 h. A sharp increase in histamine (from 68 to 297 mg/100 g of tuna) occurred between 18 and 24 h of incubation while the count rose slightly (from 2.4×10^5).

Bacterial isolates. Table 1 shows the different types of organisms isolated from decomposed skipjack tuna after 24 h at 38°C. The obligately aerobic bacteria represented only 7.5% of the microflora and did not include any histamine formers. About 24% of the isolates were obligately anaerobic clostridia, and these included nine *C. perfringens* isolates which were all histidine decarboxylase positive. Nine of the twelve facultatively anaerobic isolates were histidine decarboxylase positive, and these were gram-negative rods belonging to *Enterobacter aerogenes*, *K. pneumoniae*, *Proteus mirabilis*, and *Vibrio alginolyticus*.

TFIB. Table 2 shows the extent of growth and histamine formation by histidine decarboxylase-positive isolates inoculated in skipjack TFIB. The *C. perfringens* isolates were clearly the most active histamine formers of all bacteria tested, but they grew poorly in TFIB, even under anaerobic conditions, and did not produce much histamine. On the other hand, *E. aerogenes* grew very well in TFIB under aerobic or anaerobic conditions but formed little histamine in either case. Both *P. mirabilis* and *V. alginolyticus* grew well but produced only small amounts of histamine. By contrast, *K. pneumoniae* and *P. morganii* no. 180 produced abundant histamine after good growth in TFIB. Overall, the facultatively anaerobic isolates grew better under aerobic conditions. Generally, multiple isolates of the same species had similar histamine-producing capability.

Resting cell suspensions. Table 3 shows that the histamine-forming activity of *C. perfringens* cells was much higher than that of the facultatively anaerobic isolates tested.

TABLE 1. Bacteria isolated from decomposed skipjack tuna

Organism	No. of isolates	No. of histidine decarboxylase-positive isolates[a]
Obligately aerobic		
Micrococcus luteus	6	0
Planococcus citreus	4	0
Obligately anaerobic		
Clostridium bifermentans	1	0
C. botulinum type C	2	0
C. ghoni	7	0
C. mangenotii	11	0
C. novyi type B	1	0
C. perfringens	9	9
C. sardiniensis	1	0
Facultatively anaerobic		
Aeromonas spp.	30	0
Enterobacter aerogenes	1	1
Klebsiella pneumoniae	2	2
Pediococcus halophilus	2	0
Proteus mirabilis	3	3
Vibrio alginolyticus	23	3
V. anguillarum	31	0

[a] Of the 134 isolates studied, 18 were histidine decarboxylase positive.

DISCUSSION

Histamine distribution in decomposed tuna. During decomposition, histamine is not produced uniformly throughout the fish (16, 22). Previously we found that a gradient had formed in which histamine was highest in section no. 1 and decreased gradually in sections approaching the posterior end of the fish (12). One possible explanation for this pattern of histamine distribution is that histidine itself was not homogeneous throughout the fish. However, we found this not to be the case since histidine was

TABLE 2. Growth and histamine formation by tuna isolates incubated 48 h at 38°C in TFIB[a]

Organism	Viable count/ml		Histamine (μg/10⁶ cells)	
	Aerobic	Anaerobic	Aerobic	Anaerobic
C. perfringens				
UH-1[b]	—[c]	3.1×10^5	ND[d]	100
UH-2	—	5.4×10^4	ND	110
UH-3	—	8.6×10^4	ND	110
UH-4	—	4.6×10^4	ND	110
UH-5	—	4.2×10^3	ND	95
E. aerogenes				
UH-1	1.3×10^9	5.4×10^9	0.026	0.00072
P. mirabilis				
UH-1	3.2×10^7	3.2×10^6	0.062	6.8
UH-2	3.8×10^8	1.7×10^6	0.057	6.4
UH-3	7.3×10^8	6.2×10^6	0.057	6.9
V. alginolyticus				
UH-1	1.5×10^7	6.4×10^6	0.14	0.56
UH-2	4.0×10^7	3.0×10^6	0.14	0.56
UH-3	5.5×10^7	4.7×10^6	0.14	0.57
K. pneumoniae				
UH-1	1.5×10^8	3.0×10^7	22	29
UH-2	5.0×10^7	1.1×10^8	22	29
P. morganii no. 180[e]	8.3×10^8	5.5×10^8	2.4	2.9

[a] Inoculum levels of between 5×10^2 and 5×10^3 cells per ml were obtained from 24-h cultures grown at 38°C in Trypticase soy broth.
[b] UH, Strains isolated in this study.
[c] No growth.
[d] ND, Not detectable.
[e] From D. A. Corlett, Jr., Del Monte Research Center, Walnut Creek, Calif.

essentially uniform in fresh skipjack tuna.

We believe that the non-uniformity of histamine was due to the distribution pattern of spoilage organisms within the fish. A higher level of histamine in section no. 1 suggests the presence of a greater number of spoilage organisms released from the degenerating intestinal tract located anterior in fish. The presence of many intestinal organisms in the spoilage microflora, including *C. perfringens*, lends support to this view.

Skipjack decomposition under controlled conditions. The relationship between bacterial concentration and histamine content is difficult to interpret. In section no. 2, for example, the total anaerobic count was moderate even though the histamine level was fairly high (Fig. 1). The histamine level in any part of the fish represents the amount produced by a population of diverse organisms with groups of varying numbers. Hence, it was not possible to determine how many organisms were responsible for producing a given amount of histamine in a decomposed tuna.

Spoilage organisms. In this study, 13.4% of the microflora in decomposed skipjack tuna were histamine formers (Table 1). The most abundant histidine decarboxylase-positive isolate, *C. perfringens*, is commonly included among histamine formers (1, 9, 18, 24, 31, 33), but its presence and histamine production in decomposing fish have not been demonstrated before the present study. Of the four facultatively anaerobic, histidine decarboxylase-positive isolates shown in Table 1, all but *V. alginolyticus* have been reported previously as histamine formers in fish (1, 2, 18, 19, 24, 26, 33).

The large variety of organisms that can form histamine was illustrated by Taylor et al. (33) who studied this activity in 112 isolates representing 38 bacterial species. The diversity of histamine-forming organisms observed in scombroid fish can be attributed to differences in the species of fish, handling procedures, holding times and temperatures. In addition, the character of the microflora can be influenced by feeding habits, geographical location, season, ocean temperature, etc. (21, 29). Generally, the incidence of histamine-forming bacteria is relatively low, having been estimated at about 1.0% of the surface microflora of live fish (21).

Growth and histamine formation in TFIB.

TABLE 3. Histidine decarboxylation by resting cell suspensions of bacteria isolated from tuna[a]

Organism	Histamine formed (μg/10⁶ cells)
C. perfringens UH-1[b]	741
C. perfringens UH-2	838
K. pneumoniae UH-1	25.1
K. pneumoniae UH-2	12.4
K. pneumoniae no. T2[c]	35.4
P. morganii no. 180[d]	20.6

[a] Washed cells (5×10^7 to 4×10^9/ml) of each strain were suspended in 0.2 M sodium phosphate buffer (pH 6.0) containing 0.1% histidine. Histamine formation was measured after 24 h at 38°C. Anaerobic incubation, which did not affect significantly the activities of facultatively anaerobic strains (Table 2), was employed to permit comparison with *C. perfringens* cells. For details of cell preparation and incubation see the text.

[b] UH, Strains isolated in this study.

[c] From S. L. Taylor, Letterman Army Institute of Research, San Francisco, Calif.

[d] From D. A. Corlett, Jr., Del Monte Research Center, Walnut Creek, Calif.

TFIB alone was not suitable for comparing histamine formation by the histidine decarboxylase-positive isolates obtained in this study. On the basis of results shown in Table 2, *C. perfringens*, for example, would not be considered a potential tuna spoilage organism. Its poor growth in TFIB probably resulted from the mild acidity (pH 6.0) which is inhibitory to *C. perfringens*. Nevertheless, the presence of *C. perfringens* isolates in the spoilage microflora (Table 1) and the active histidine decarboxylase of its whole cell suspensions suggest a potentially significant role in anaerobic decomposition of tuna.

The effect of anaerobic conditions on histamine formation is not clear. Kimata (21) reported that *P. morganii* produced more histamine under anaerobic conditions, while Ferencik (10) reported the opposite for *Hafnia* spp. The data shown in Table 2, however, indicate that histamine formation is dependent upon the amount of growth rather than on the growth conditions per se.

Several workers have described histaminase activity among histidine-decarboxylating organisms (2, 7, 10, 18, 33). To date, we have not investigated the effect of bacterial histaminases on histamine formation in whole tuna or in the experimental systems described in this study.

Histamine formation by resting cells. The role of anaerobic bacteria in histamine production in scombroid fish has not been examined previously. Organisms such as *C. perfringens* are present in the intestinal microflora of fish and are able to grow and produce histamine in the anaerobic environment of tuna muscle tissue. In this study, one-half of the histidine decarboxylase-positive bacteria isolated were *C. perfringens*. Resting cell suspensions of the *C. perfringens* isolates had the highest histidine decarboxylase activity among the six strains tested (Table 3). The low histidine decarboxylase activity of *K. pneumoniae* and *P. morganii* cells probably is offset by their ability to grow well in tuna. Thus, the total amount of histamine produced by these bacteria would exceed that formed by slower-growing *C. perfringens* (Table 2).

Further study of the contribution of other anaerobic bacteria to histamine formation and decomposition in other tuna appears worthwhile.

Significance of results. A large assortment of bacteria have been associated with histamine formation in various scombroid fish (1, 2, 18, 21, 22, 25, 26, 31, 33), including several of the bacterial species reported in this study (Table 1). We found a variety of bacteria in the loin tissue of a decomposed skipjack tuna, and 5 of the 18 species isolated were potential histamine producers. It is likely that additional histamine-forming species might have been isolated if more than one sample of spoiled fish were tested. Histamine-producing organisms are present in the skin, gills, and intestines of many fish and are considered to be part of the normal microflora (1, 21).

Our results and those of other investigators (1, 22, 25, 26, 33) suggest that the histamine in scombroid fish may be produced by several species of bacteria during decomposition. Our data also show that histamine can be produced under anaerobic conditions (loins) and also may be formed by obligately anaerobic bacteria (clostridia). *K. pneumoniae*, which produced ample histamine under anaerobic and aerobic growth conditions, also has been cited in several reports (22, 25, 26). Other histamine-forming bacteria may be found when different species of scombroid fish decompose. For example, *P. morganii*, an active histamine producer (21, 25, 26, 33), was not recovered from skipjack tuna in our study but may be found in other fish or under different spoilage conditions.

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