Identification of Bacteria Crucial to Histamine Accumulation in Pacific Mackerel during Storage†

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ABSTRACT

Bacterial growth and histamine formation in Pacific mackerel during storage at 0, 4, 15, and 25°C were monitored. To identify bacterial species contributing to histamine formation, several groups of bacteria were isolated by using selective media under temperatures corresponding to the various storage conditions. Initially, low counts of bacteria were found in the gill, skin, and intestine of fresh fish, and only weak histamine formers were found in the gill. Histamine was found in the muscles when fish were stored above 4°C, and aerobic plate counts reached 10⁶ CFU/g. When fish became unsuitable for human consumption by abusive storage, toxicological levels of histamine were always found. The highest level of histamine formed was 283 mg/100 g in 2 days. The optimum temperature for supporting growth of prolific histamine formers was 25°C. The most prolific and prevalent histamine former was Morganella morganii, followed by Proteus vulgaris, both of which were isolated on violet red bile glucose (VRBG) agar. At 15°C, a significant level of histamine was still produced in fish muscle, although prolific histamine formers were less frequently detected than at 25°C. The isolates on thiosulfate citrate bile salts sucrose (TCBS) agar were weak histamine formers and identified as Vibrio parahaemolyticus and Vibrio alginolyticus. At 4°C, less than 57.4 mg/100 g of histamine was found in fish stored for 14 days. Most isolates were natural bacterial flora in the marine environment and identified as weak histamine formers. At 0°C, neither histamine former nor histamine production was detected up to 14 days of storage.

Scombroid poisoning is a foodborne chemical intoxication. Histamine is the main causative agent in scombroid poisoning, although other biogenic amines, such as cadaverine and putrescine, have been reported to play a synergistic role with histamine (35). Histamine is a chemical hazard monitored by the Food and Drug Administration for safety of seafood products (16). It can be present at high levels in fresh fish without any signs of decomposition. In general, time and temperature abuse of fish results in histamine accumulation, although the severity of symptoms varies depending on the individual’s sensitivity to ingested histamine (34). Scombroid fish containing high levels of free histidine in the muscle are susceptible to histamine formation and are most frequently implicated in outbreaks of scombroid poisoning (38).

Histamine is rarely found in fresh fish (25, 28). Improper handling and storage of fish can induce histamine formation by the proliferation of bacteria possessing histidine decarboxylase, the enzyme responsible for the conversion of histidine to histamine (35). Several species of enteric bacteria are the representative histamine formers in fish, although many other bacteria, including Lactobacillus spp., Bacillus spp., and Clostridium spp., contain the enzyme (2, 5, 29, 37, 38, 44). The most prolific histamine former reported is Morganella morganii, followed by Klebsiella spp. and Enterobacter spp. (2, 41). Other enteric bacteria, i.e., Hafnia alvei, Citrobacter freundii, and Serratia spp., are reported as weak histamine formers (25, 28). Other types of histamine formers isolated from fish are psychrophilic psychrotrophs. Pseudomonas spp., Photobacterium spp., Aeromonas spp., and Vibrio alginolyticus have often been reported as histamine formers capable of producing histamine at refrigeration temperature (5, 30, 37). Among them, Photobacterium phosphoreum isolated from mackerel has been reported as an important histamine former (30).

The dominant bacterial flora in fish can change depending on handling and storage conditions. Since most prolific histamine formers are mesophiles, temperature is a critical factor affecting histamine formation in fish (24). In many studies, the optimal storage temperature reported for histamine production is 20 to 25°C (1, 13, 23). Low temperature storage at 0°C or below can effectively control their growth and histamine formation (23, 33, 34). In a few studies, histamine was detected during storage at 0°C, but only after fish became unacceptable for human consumption (9, 12, 22, 28).

The effect of temperature on histamine formation between 2 and 10°C is not clear. At these temperatures, histamine formation is assumed to be formed due to the growth of psychrotrophic or psychrophilic bacteria (9, 12). However, these are mainly weak histamine formers; thus, their contribution to histamine accumulation may not be as...
significant as that of mesophilic histamine formers. Therefore, the objective of this study was to monitor Pacific mackerel for product safety under various storage conditions. In this study, histamine production and bacterial growth were monitored on fish under storage at 0 to 25°C. Several groups of bacteria were isolated using selective media, and the confirmed histamine formers were identified to species.

MATERIALS AND METHODS

Reagents. Dowex 1-X8, histamine dihydrochloride, o-phthalaldehyde, and tetramethyl-p-phenylenediamine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, Mo.) Kovacs' reagent was obtained from Difco Laboratories (Detroit, Mich.).

Media. Trypticase soy broth was purchased from BBL Co. (Cockeysville, Md.). Standard plate count agar, violet red bile glucose (VRBG) agar; pseudomonas isolation (PI) agar; thiosulfate citrate bile salts sucrose (TCBS) agar, and tryptone broth were obtained from Difco.

Samples. Pacific mackerel (Scomber japonicus) were commercially harvested off the Oregon Coast. On the day fish were delivered to the seafood processing company (Astoria, Ore.), Pacific mackerel, ranging from 550 to 600 g, were obtained as samples. The fishing trips usually lasted less than 8 h, and the mackerel samples were in excellent quality. The fish were transported in ice to Oregon State University-Seafood Laboratory, Astoria, Ore. Whole fish were immediately used for enumeration of initial bacterial counts and isolation of histamine formers. The remaining fish were separately placed in sterile plastic bags and sealed. Five and three fish were stored at 15 and 25°C, respectively, and seven fish each were stored at 0 and 4°C. Samples were taken every 24 h from fish stored at 15 and 25°C and every 48 h from those stored at 0 and 4°C for aerobic plate counts (APC), histamine analysis, and isolation of histamine formers.

Sample treatment and APC. Skin, gill, and intestine of fresh fish were taken for APC. A sterile aluminum foil template (4 by 5 cm) was placed on the surface of skin, and the area of template was swabbed with sterile cotton. The cotton swab was inoculated into 5 ml Trypticase soy broth (32). Aseptically, 10 g of the gill and intestine was removed and blended with 90 ml of peptone water (0.1%). For sampling muscles, the skin (3 by 8 cm²) was excised. Immediately, 10-g portions of dorsal muscles were taken, weighed into a sterile jar, and blended with 90 ml of peptone water (0.1%). APC was carried out in duplicate according to the standard plate method (14). Each dilution was mixed with selective media for screening histamine formers. The selective media used for target bacteria were VRBG agar for enteric bacteria; TCBS agar for vibrios; and PI agar for pseudomonads. Colonies on each plate were picked based on their morphological characteristics, such as color, size, opacity, and form, and inoculated into tryptic soy agar slants for histamine analysis.

Histamine formation by the isolates. Bacterial isolates were inoculated into Trypticase soy broth supplemented with 1% histidine and incubated at 37°C for 24 h (41). One milliliter of bacterial culture was mixed with 9 ml of methanol. Histamine content in culture broth was determined in duplicate by the AOAC fluorometric method (3) as described above.

Identification of histamine formers. Bacterial isolates confirmed to produce histamine were identified by using the Vitek instrument (bioMérieux Vetek, Inc., Hazelwood, Mo.) according to the protocol recommended by the manufacturer. Each isolate was inoculated in Trypticase soy agar slants and incubated at 37°C for 18 h. Gram staining and oxidase tests required for the selection of adequate identification cards were carried out using the standard method (10). Each culture was suspended in phosphate saline buffer at the concentration range determined by the Vitek colorimeter and placed in a Vitek identification card. The bacterial identification was automatically carried out by the Vitek system.

Additional biochemical tests were carried out for definitive species identification. For Pseudomonas species, gelatin utilization was tested by liquefication of gelatin medium (10). One loopful of culture was inoculated into gelatin medium and incubated at 22°C for 10 days. To differentiate two species of Proteus, an indole test was carried out using the standard method (10). One loopful of culture was inoculated into tryptone broth and incubated at 37°C for 24 h. To the bacterial culture, 0.1 ml of Kovacs' reagent was added, and change of color was monitored.

RESULTS

Initial bacterial loads and isolation of histamine formers from fresh Pacific mackerel. The Pacific mackerel samples received were in excellent condition, as judged by the sensory qualities, such as the appearance of fish, muscle texture, and odor. All samples had bright eyes, red gills, and firm texture. Bacteria were initially found in the gill and intestine and on the skin of the fresh fish, but rarely in the muscle. The APC of the gill and skin were 1.5 × 10⁵ CFU/g and 1.6 × 10⁴ CFU/cm², respectively (Table 1). A relatively low APC, 2.6 × 10⁵ CFU/g, was detected in the intestine. However, prolific histamine formers were not detected in any of the samples tested. Most isolates on the selective media were nonhistamine formers, and a few from the gill were weak histamine formers. Among 15 isolates obtained on TCBS agar, seven strains produced 20 to 50 ppm histamine in culture broth (data not shown). The identified species were Vibrio parahaemolyticus, V. alginolyticus, and Aeromonas hydrophila.
TABLE 1. Counts of total aerobic bacteria and isolation of histamine formers from fresh Pacific mackerel

<table>
<thead>
<tr>
<th>Origin of isolates</th>
<th>APC (CFU/g or cm²)</th>
<th>VRBG</th>
<th>PI</th>
<th>TCBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>1.5 x 10⁵</td>
<td>0/31</td>
<td>0/14</td>
<td>7/15</td>
</tr>
<tr>
<td>Skin</td>
<td>1.6 x 10⁴</td>
<td>0/18</td>
<td>0/18</td>
<td>0/16</td>
</tr>
<tr>
<td>Intestine</td>
<td>2.6 x 10²</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Muscle</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Histamine production by isolates was confirmed by the AOAC fluorometric method.

b Target groups of bacteria were isolated using selective media.

c ND, not detected.

Changes in bacterial counts and histamine contents at various temperatures. Bacterial counts rapidly increased when fish were exposed to ambient temperature (25°C). APC reached 4.1 x 10⁶ CFU/g in 1 day (Fig. 1). Fish appeared spoiled, and off-odor was detected. Cloudy slime appeared on the surface of skin. Muscle turned opaque, and its texture became soft. Belly was burst, and intestine wall was completely dissolved by autolysis. APC reached the maximum level, 9.5 x 10⁷ CFU/g, in 2 days but decreased thereafter. The pattern of quality changes at 15°C was slightly different from 25°C. The intestinal wall of fish remained intact, and the muscle consistently showed translucent-red color. Off-odor, cloudy slime on the surface of skin, and belly burn were detected in 2 days. APC gradually increased and reached 2.8 x 10⁷ CFU/g in 4 days. As storage temperature decreased, the rate of bacterial growth was lower, and the quality changes progressed more slowly. At 4°C, APC reached 6.8 x 10⁵ CFU/g in 8 days of storage. By then, fish were apparently decomposed and unsuitable for human consumption. Off-odor and belly burn in fish were evident when APC reached 9.3 x 10⁶ CFU/g in 10 days. At 0°C, APC slowly increased and did not exceed 10⁶ CFU/g for 14 days. Fish developed cloudy slime on the surface of skin and slight off-odor in 10 days.

The optimal temperature for histamine formation in fish muscles was 25°C (Fig. 2). The histamine content exceeded 5 mg/100 g in 1 day. It increased rapidly and reached the highest level, 286 mg/100 g, in 2 days when spoilage was apparent. At 15°C storage, the histamine content increased slightly (14.0 mg/100 g) in 2 days, reaching 197 mg/100 g in 5 days. Both the APC and histamine content at 15°C were lower than 25°C. At 4°C, histamine was not produced in 6 days of storage (<1 mg/100 g), and the fish was still acceptable for human consumption. Histamine content began to increase thereafter and accumulated up to 57.4 mg/100 g in 14 days after gross spoilage. At 0°C, a negligible level of histamine (<1 mg/100 g) was formed in 14 days of storage.

Identification of prolific histamine formers during storage at 25°C. A total of 1,200 strains were isolated from the muscles of Pacific mackerel during storage by using selective media. Prolific histamine formers producing >1,000 ppm histamine at 37°C were mostly isolated on VRBG agar from fish stored at 25°C. The identified species were M. morganii and Proteus vulgaris. Bacterial count on VRBG agar was still low on day 1 (Fig. 3). Only one strain of M. morganii was isolated on VRBG plate (Table 2). M. morganii was frequently isolated with the progression of fish spoilage. Ten strains were isolated on day 2 and 17 strains on day 3. All the isolates produced a significant level of histamine at 37°C, ranging from 3.209 to 3.527 ppm, in Trypticase soy broth supplemented with 1% histidine. Another prolific histamine former, P. vulgaris, produced 2,985 to 3,488 ppm histamine in culture broth at 37°C. However, it was less frequently isolated than M. morganii. Four isolates of P. vulgaris were detected on day 2, and six strains were isolated on day 3. In general, morphological characteristics of P. vulgaris on VRBG agar were not significantly different from those of M. morganii. Typically, both M.
V. parahaemolyticus and P. vulgaris colonies on VRBG agar were small (2 to 3 mm in diameter), smooth, translucent, purple with or without cloudy zone, and slightly flattened with an opaque center. Other enteric bacteria were rarely isolated. Only one strain of Enterobacter aerogenes, identified as a weak histamine former, was isolated on day 2.

Weak histamine formers were frequently isolated on TCBS agar. A rapid increase in bacterial counts was shown on TCBS agar in 1 day. The highest bacterial count was also detected on TCBS agar among all the selective media tested on day 1 (Fig. 3). Identified histamine formers were V. parahaemolyticus and V. alginolyticus, which were isolated from the gill of fresh fish in this study (Table 2). However, bacterial counts on TCBS agar did not increase rapidly after the first day, and they were always lower than those on VRBG agar. V. parahaemolyticus and V. alginolyticus were frequently isolated as weak histamine formers during 3 days of storage. Among the selective media tested, the lowest bacterial count obtained was from PI agar (Fig. 3). Bacterial counts reached $10^4$ CFU/g in 3 days of storage. Most isolates produced negligible amounts of histamine in culture broth and thus were classified as nonhistamine formers.

Identification of dominant histamine-forming bacterial flora during storage at 0, 4, and 15°C. The highest bacterial counts were detected on VRBG agar among all the tested media during storage at 15°C (Fig. 3). Bacterial counts on selective media were low on day 1 for fish samples stored at 15°C, and histamine formers were not isolated from the samples (Table 3). M. morganii was first isolated on day 2. A total of 11 strains were isolated during 5 days of storage. Minor bacterial species identified were Edwardsiella hoshinae, Pseudomonas putida, and H. alvei. They were confirmed as weak histamine formers. Bacterial counts on TCBS agar gradually increased over the storage time. V. parahaemolyticus and V. alginolyticus were isolated only from fish stored for 3 days (Table 3). In general, M. morganii was less frequently isolated, and the detection rate of vibrios decreased more substantially at 15°C than at 25°C.

At 4°C, the highest bacterial counts were obtained with VRBG agar (Fig. 4). Although higher bacterial counts were initially detected on PI agar than on VRBG agar at the beginning of storage, their counts did not change much after 6 days of storage. Bacterial growth on TCBS was first detected in fish samples stored for 8 days, and only a slight increase in bacterial count was observed thereafter. Histamine formers were not isolated up to 6 days of storage. They were isolated from the 8-day stored fish that contained 11.3 mg/100 g histamine (Table 4). All isolates were weak histamine formers, and their detection rates were low. Although they were isolated only on VRBG agar, the identified histamine formers were a part of naturally existing bacterial flora in seawater. Actinobacillus ureae was isolated from fish stored for 8 and 10 days, and one strain of P. putida was isolated from fish stored for 10 days. A. hydrophila was frequently isolated at the end of the storage test.

V. alginolyticus and Photobacterium damsela were also isolated as histamine formers. At 0°C, no histamine former was isolated during storage up to 14 days. The highest bacterial count was obtained from PI agar, indicating changes in the dominant culturable bacterial flora (Fig. 4). Few colonies grew on VRBG agar, which was the most effective medium for the isolation of histamine formers during storage above 4°C.

**DISCUSSION**

Quality changes and product safety of Pacific mackerel during storage. Shelf life of raw seafoods depends on several factors: storage conditions (time, temperature, presence and concentration of gases, and relative humidity of the environment), intrinsic factors of fish (species, age, size, fat content, feeding, and physiological status), and number of initial microflora (18, 19). Handling fresh mackerel can be a problem due to its soft flesh, high lipid content, and delicate skin (22).

In this study, we demonstrated that improper storage of mackerel resulted in the substantial deterioration of fish quality in a short period of time. The rapid quality deterioration was accompanied by bacterial proliferation at ambient temperature, 25°C, within 1 day, indicating that natural bacteria existing in the gill, skin, and intestine had spread into the muscle rich in nutrients. The quality change of mackerel progressed faster than that of larger fish, such as tuna and mahi-mahi. When albacore were stored at 25°C, APC reached only $10^7$ CFU/g in 7 days (23). With mahi-mahi, the decomposition was also slow at 21°C (7). The APC reached $10^5$ CFU/g in 2 days of storage, and histamine was not detected. The highest level of histamine, 234 mg/100 g, was found in 4 days of storage. However, the highest levels of APC and histamine content were detected in mackerel stored for only 2 days (Figs. 1 and 2).

The general recommendation of microbiological limits in fish is $5 \times 10^6$ CFU/g (21). It is well documented that histamine is a health hazard if present in fish muscle at levels higher than 50 mg/100 g (16). When APC reached $10^5$ CFU/g during storage above 4°C, mackerel was unacceptable for human consumption (Fig. 1). The levels of histamine produced exceeded 5 mg/100 g, the Food and Drug Administration guideline for scombroid fish (15). A health hazard level of histamine (>50 mg/100 g) accumulated after APC reached $>10^3$ CFU/g, although the levels were variable depending on the storage temperatures. Overall, mackerel was a highly perishable fish. Histamine above the health hazard level was produced even during storage at 4°C. However, it occurred only after fish were completely decomposed and unsuitable for human consumption, as previously reported with other fish species, i.e., tuna (28) and albacore (23).

**Presence of histamine formers in fresh fish.** The initial bacterial loads on the gill and skin of fresh Pacific mackerel were not greatly different from previous reports of $10^2$ to $10^8$ CFU/cm² on the skin and $10^2$ to $10^5$ CFU/g on the gills (27). Gennari et al. (18) reported that $10^4$ CFU/cm² and $10^5$ CFU/g of APC were detected on the skin and
gills of fresh sardine, respectively. Bacterial counts in fresh sardine were $2.5 \times 10^6$ CFU/cm$^2$ on the skin; $1.3 \times 10^3$ CFU/g on the gills; and $3.1 \times 10^4$ CFU/g in the intestine (2). However, the bacterial counts in the intestine were lower than the reported data, $>10^7$ CFU/g in feeding fish (27).

Histamine formers may constitute a minority of the natural microflora, thus making it difficult to detect in fresh fish (39). However, it is necessary to monitor the population and distribution of prolific histamine formers in fresh fish. Many researchers suspect the presence of histamine formers in the gut and gill of fresh fish, since histamine is usually detected in fish at higher levels in tissues adjacent to the gills or intestines (28, 40). When histamine formers were isolated from fresh albacore after the enrichment procedure, most of them were weak enteric histamine formers, and only few species—i.e., *Citrobacter braakii* and *E. aerogenes*—were prolific formers (25).

In this study, the presence of prolific histamine formers was not detected in fresh mackerel, and all the natural bacterial species isolated were confirmed as weak formers. However, prolific histamine formers were frequently isolated from temperature-abused (>15°C) fish for a short period of time (Tables 2 and 3). Therefore, we suspect that the initial population of prolific histamine-producing bacteria in fresh fish was too low (<10$^6$ CFU/g) to be isolated by the culture method. Although they are present at negligible levels in fresh fish, they may proliferate, synthesize histidine decarboxylase, and produce histamine when fish are left at elevated temperatures.

**M. morganii** as the main contributor of histamine formation in fish. *M. morganii* has frequently been isolated from various types of temperature-abused fish—i.e., skipjack tuna (32), tuna (*Thunnus thynnus*) (28), albacore (24), mahi-mahi (17), mackerel (29), sardine (2), and Spanish salted semipreserved anchovies (36).
TABLE 2. Identification of histamine formers from the muscles of Pacific mackerel during storage at 25°C

<table>
<thead>
<tr>
<th>Storage day(s)</th>
<th>Strain isolated</th>
<th>Number isolated</th>
<th>Medium used&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Histamine&lt;sup&gt;b&lt;/sup&gt; (ppm) produced in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vibrio parahaemolyticus</td>
<td>3</td>
<td>TCBS</td>
<td>25.8–230 (125 ± 102)</td>
</tr>
<tr>
<td></td>
<td>Morganella morganii</td>
<td>1</td>
<td>VRBG</td>
<td>3.191</td>
</tr>
<tr>
<td></td>
<td>Vibrio alginolyticus</td>
<td>1</td>
<td>TCBS</td>
<td>88.2</td>
</tr>
<tr>
<td>2</td>
<td>M. morganii</td>
<td>10</td>
<td>VRBG</td>
<td>3,222–3,413 (3,308 ± 62.4)</td>
</tr>
<tr>
<td></td>
<td>V. parahaemolyticus</td>
<td>5</td>
<td>TCBS</td>
<td>98.2–323 (178 ± 95.2)</td>
</tr>
<tr>
<td></td>
<td>Proteus vulgaris</td>
<td>4</td>
<td>VRBG</td>
<td>3,272–3,345 (3,299 ± 31.8)</td>
</tr>
<tr>
<td></td>
<td>V. alginolyticus</td>
<td>4</td>
<td>TCBS</td>
<td>41.5–78.0 (57.3 ± 16.2)</td>
</tr>
<tr>
<td></td>
<td>Enterobacter aerogenes</td>
<td>1</td>
<td>VRBG</td>
<td>23.4</td>
</tr>
<tr>
<td>3</td>
<td>M. morganii</td>
<td>17</td>
<td>VRBG</td>
<td>3,209–3,527 (3,306 ± 93.0)</td>
</tr>
<tr>
<td></td>
<td>P. vulgaris</td>
<td>6</td>
<td>VRBG</td>
<td>2,985–3,488 (3,234 ± 162)</td>
</tr>
<tr>
<td></td>
<td>V. alginolyticus</td>
<td>5</td>
<td>TCBS</td>
<td>63.9–122 (85.8 ± 24.3)</td>
</tr>
<tr>
<td></td>
<td>V. parahaemolyticus</td>
<td>5</td>
<td>TCBS</td>
<td>11.8–114 (51.4 ± 44.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Target group of bacteria was isolated by using selective medium.

<sup>b</sup> Histamine production at 37°C by isolates was confirmed by the AOAC fluorometric method.

as the most prolific histamine former capable of producing >1,000 ppm histamine in culture broth. However, the isolation rate of M. morganii varied depending on the fish species stored at temperatures above 25°C. M. morganii was identified as the most prolific histamine former in sardines, but only seven strains were isolated from samples stored at ambient temperature (25°C) (2). The most frequently isolated histamine-producing bacteria were Proteus spp. Twenty-three strains out of 28 Proteus strains isolated were prolific histamine formers. Only 9% of histamine formers were M. morganii in mahi-mahi stored at 32°C (17). Eight strains of M. morganii and one strain of P. mirabilis were identified as prolific histamine producers (>1,000 ppm). V. alginolyticus comprised 90% of the mesophilic isolates, but most isolates (97 strains) did not produce histamine. Only 18 strains of V. alginolyticus were weak histamine formers (<100 ppm).

M. morganii played the major role in histamine accumulation in Pacific mackerel during storage at elevated temperature (>15°C). Histamine accumulation in fish muscles was proportional to the proliferation of M. morganii during the first 2 days. Although more M. morganii strains were isolated on day 3 than on day 2 during storage at 25°C, the histamine content in fish muscle was lower on day 3 than on day 2. It was reported that diamine oxidase is primarily responsible for the metabolism of histamine by catalyzing oxidative deamination of histamine (35, 40). Diamine oxidase was found in fish muscle (20). Therefore, it has been presumed that the decrease in histamine content in fish resulted from metabolism of histamine.

Using VRBG agar, other enteric bacteria—i.e., Klebsiella oxytoca, Klebsiella pneumoniae, Serratia liquefaciens, Enterobacter cloacae, and C. freundii, which are commonly isolated from temperature-abused fish (>25°C)—were not isolated in this study. One strain each of E. aerogenes, E. hoshinae, and H. alvei was isolated on VRBG agar from fish stored at 15 or 25°C. Only P. vulgaris was frequently isolated on VRBG agar and produced the comparable level of histamine to M. morganii in culture broth.

Effect of temperature on growth of M. morganii and its histamine production has been well studied. Generally,

TABLE 3. Identification of histamine formers from the muscles of Pacific mackerel during storage at 15°C

<table>
<thead>
<tr>
<th>Storage day(s)</th>
<th>Strain isolated</th>
<th>Number isolated</th>
<th>Medium used&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Histamine&lt;sup&gt;b&lt;/sup&gt; (ppm) produced in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Morganella morganii</td>
<td>3</td>
<td>VRBG</td>
<td>3,324–3,367 (3,348 ± 22.2)</td>
</tr>
<tr>
<td></td>
<td>Edwardsiella hoshinae</td>
<td>1</td>
<td>VRBG</td>
<td>33.7</td>
</tr>
<tr>
<td>3</td>
<td>M. morganii</td>
<td>4</td>
<td>VRBG</td>
<td>3,201–3,340 (3,304 ± 68.9)</td>
</tr>
<tr>
<td></td>
<td>Vibrio parahaemolyticus</td>
<td>3</td>
<td>TCBS</td>
<td>42.9–142 (76.7 ± 56.5)</td>
</tr>
<tr>
<td></td>
<td>Vibrio alginolyticus</td>
<td>1</td>
<td>TCBS</td>
<td>837</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas putida</td>
<td>1</td>
<td>VRBG</td>
<td>159</td>
</tr>
<tr>
<td>4</td>
<td>M. morganii</td>
<td>2</td>
<td>VRBG</td>
<td>3,203–3,400</td>
</tr>
<tr>
<td></td>
<td>Proteus vulgaris</td>
<td>1</td>
<td>VRBG</td>
<td>3,225</td>
</tr>
<tr>
<td>5</td>
<td>M. morganii</td>
<td>2</td>
<td>VRBG</td>
<td>3,302–3,349</td>
</tr>
<tr>
<td></td>
<td>Hafnia alvei</td>
<td>1</td>
<td>VRBG</td>
<td>148</td>
</tr>
</tbody>
</table>

<sup>a</sup> Target group of bacteria was isolated by using selective medium.

<sup>b</sup> Histamine production at 37°C by isolates was confirmed by the AOAC fluorometric method.

<sup>c</sup> ND, not detected.
FIGURE 4. Changes in bacterial counts of Pacific mackerel muscle during storage at 0 and 4°C as determined by VRBG, TCBS, and PI agar. Bacterial counts were determined in duplicate by the standard pour plate method.

the temperature range of 25 to 37°C is optimal for histamine production in culture broth with this bacterium (8, 11). The minimum temperature to produce toxicological levels of histamine in culture broth is 15°C (8, 24, 26). M. morganii showed the typical characteristics of mesophilic bacteria, in that bacterial growth as well as histamine formation were inhibited by storage below 4°C (4, 24, 26). These characteristics were well reflected when M. morganii was isolated from Pacific mackerel stored at different temperatures in this study. The isolation rate of M. morganii decreased as the storage temperature decreased, regardless of the length of storage. M. morganii was not isolated at 4°C, coinciding with the low levels of histamine found in fish muscle. However, when M. morganii previously incubated at 25°C for 23 h was placed at 5°C, histamine continually accumulated, increasing from 800 to 2,700 ppm in culture broth in 5 days of incubation (26). Baranowski et al. (6) also reported that histidine decarboxylase already produced by bacteria could still convert histidine to histamine even after inactivation of the cells. In this study, growth of bacteria including M. morganii accelerated histamine accumulation at ambient temperature (25°C) even for a short period of storage. Therefore, any delay in proper chilling would allow continuous histamine accumulation to high levels in fish muscle. It has been recommended that fish susceptible to histamine formation be chilled below 4°C in 4 h immediately after harvest on the vessel and that the internal temperature of fish muscle be maintained in a proper chilling system throughout storage and distribution to prevent scombroid poisoning (16).

Histamine production by natural bacterial flora associated with the marine environment. Many of the natural bacterial flora in the marine environment are psychrophils or psychrophiles. Therefore, histamine-producing psychrotrophic bacteria have been studied to control histamine formation during storage of fish at refrigeration temperature or below (30, 35). In this study, several species of

<table>
<thead>
<tr>
<th>Storage day(s)</th>
<th>Strain isolated</th>
<th>Number isolated</th>
<th>Medium used&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Histamine&lt;sup&gt;b&lt;/sup&gt; (ppm) produced in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>Actinobacillus ureae</td>
<td>2</td>
<td>VRBG</td>
<td>248–256</td>
</tr>
<tr>
<td>10</td>
<td>Pseudomonas putida</td>
<td>1</td>
<td>VRBG</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>A. ureae</td>
<td>1</td>
<td>VRBG</td>
<td>154</td>
</tr>
<tr>
<td>12</td>
<td>Aeromonas hydrophila</td>
<td>1</td>
<td>VRBG</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Vibrio alginolyticus</td>
<td>1</td>
<td>VRBG</td>
<td>111</td>
</tr>
<tr>
<td>14</td>
<td>A. hydrophila</td>
<td>3</td>
<td>VRBG</td>
<td>72.9–182 (145 ± 62.4)</td>
</tr>
<tr>
<td></td>
<td>Photobacterium damsela</td>
<td>1</td>
<td>VRBG</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>V. alginolyticus</td>
<td>1</td>
<td>VRBG</td>
<td>8.83</td>
</tr>
</tbody>
</table>

<sup>a</sup> Target group of bacteria was isolated by using selective medium.

<sup>b</sup> Histamine production at 37°C by isolates was confirmed by the AOAC fluorometric method.

<sup>c</sup> ND, not detected.
natural bacteria were the main histamine producers during storage at 4°C, the limiting temperature for the growth of the enteric bacteria. However, they were weak formers and isolated only with the extended storage.

*V. parahaemolyticus* was the only mesophilic bacteria identified. Although this species was originally isolated from fresh fish, its proliferation was notable only at 25°C due to its cold sensitivity (31). *V. alginitolyticus* was the only species isolated during storage at 4 to 25°C, showing the characteristics of psychrotrophic bacteria. Morii et al. (30) reported that *P. phosphoreum* may be principally responsible for histamine formation in scombroid fish at low temperatures. However, only one strain of *P. damselflora* was isolated in this study, and it produced 112 ppm histamine in culture broth. *Aeromonas* spp. are widely distributed in the aquatic environment and have been isolated from various seafoods and dairy products during refrigerated storage (43). Most species are psychrophils and reported as emerging foodborne pathogens associated with septicemia and gastroenteritis in humans (42). Although *A. hydrophila* has not been frequently reported as a histamine former, it was isolated from both fresh and spoiled fish at 4°C in this study. *Pseudomonas* spp. were not often isolated as histamine formers. Most isolates from Pl agar were confirmed as nonformers. Only two strains were isolated on VRBG agar during storage at 4 and 15°C, and their histamine-producing capacities were insignificant. Similar results were obtained with fluorescent and nonfluorescent pseudomonads when tested for histamine production (37). They produced the low levels of histamine during incubation in the histidine decarboxylase broth at 21°C for 48 h. Overall, the naturally present bacteria could proliferate at refrigeration temperature, but their contribution to histamine accumulation was negligible.

**CONCLUSION**

Temperature greatly influenced the growth of various types of bacterial flora and, consequently, histamine accumulation in Pacific mackerel during storage. Histamine accumulation was conspicuous with prevalence of prolific histamine formers during storage at ambient temperature (25°C). *M. morganii* was the main contributor to histamine formation in fish muscle during storage at 15 to 25°C. Growth of prolific histamine formers was controlled by storage of fish at 4°C or below. At 4°C, natural bacterial flora were the main histamine formers rather than *M. morganii* and other enteric bacteria. Although the natural bacterial flora were weak histamine formers, a hazardous level of histamine was detected in fish muscle only after fish became unsuitable for human consumption. At 0°C, no histamine was found, and the growth of histamine formers was effectively controlled.

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**REFERENCES**


logical analysis: principles and specific application, 2nd ed. University of Toronto Press, Buffalo, N.Y.


