Microbiological Flora of Aquacultured Hybrid Striped Bass

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ABSTRACT

Aquacultured hybrid striped bass were examined for quantity and quality of microflora. Plate counts were performed at three temperatures (7, 22, and 35°C) under aerobic and anaerobic conditions. Bacterial loads on the skin, gills, and intestines were similar to those reported for wild fish. Plate counts performed at 22°C yielded the highest counts and the greatest variety of species. The predominant groups of bacteria isolated were *Aeromonas* spp. (27%), coryneforms (14%), *Pseudomonas* spp. (12%), *Flavobacterium/Cytophaga/Sphingobacterium* group (8%), *Pleisomonas shigelloides* (7%), *Bacillus* spp. (7%), and *Enterobacteriaceae* (6%). Human foodborne pathogens *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Vibrio*, *Aeromonas*, *Flavobacterium*, *Cytophaga*, *Pseudomonas*, and *Acinetobacter*. Aerobic plate counts ranged from $5 \times 10^3$ to $6.3 \times 10^7$ CFU/g.

Fish cultured in ponds are subject to bacterial contamination from contact with the environment, feed, birds, animals, and humans; the crowded conditions of intensive culture could result in rapid spread of pathogens and high bacterial loads on the fish. The purpose of this study was to determine the numbers and species of bacteria found on farm-raised striped bass in Maryland. Special attention was given to detecting bacteria that can cause foodborne illness in humans.

MATERIALS AND METHODS

Collection of samples

Hybrid striped bass were collected from commercial, freshwater ponds on Maryland’s Eastern shore. Farm 1 has above grade, 1/2 acre (ca. 2,400 hectares) ponds; water is drawn from 150-ft (4,572 cm) deep wells fed by the Agua Aquifer. Both ponds contained about 3,000 fish, 1.5 years old. Pond 7, Farm 2, is 1.25 acres (ca. 5,000 hectares), below grade, and relied on water from wells and the Wye River; pond 1 contained about 3,000 fish, 1.5 years old. Ponds from Farm 3 were 1 acre (ca. 4,800 hectares), above grade, and relied on water from wells and the Wye River; pond 1 contained about 3,500 fish, 2 years old, and pond 4 had about 2,800 fish of the same age.

In each round, six fish were sampled from seines, cages, or by hook and line. The method of capture, location of sampling, and dates of sampling are listed in Table 1. Fish were removed from the seines and cages using dip nets. All handling after capture was done with sterile gloves. Fish were immediately transferred to individual sterile bags and buried in ice. All fish were obtained between 7 a.m. and 9 a.m. Processing of samples began within 3 h of capture.

Water samples were collected from approximately 1 ft (30.48 cm) below the surface of each pond using a Grab sampler (Wheaton, Millville, NJ) and sterile bottle. Samples were immediately iced for transport and examined within 5 h of collection.

Microbiological analyses

Fish were examined in a laminar flow hood. The six fish were randomly divided into two replicate samples of 3 fish each. Surface samples were obtained by swabbing a 10 cm x 1-cm area on the side of each fish using a calcium alginate swab (Calgiswab, Spectrum Laboratories, Los Angeles, CA); gills were sampled by swabbing a 2 cm x 1-cm area. Swabs from each replicate group...
TABLE 1. Collection of samples.

<table>
<thead>
<tr>
<th>Date</th>
<th>Farm</th>
<th>Pond</th>
<th>Method of harvest</th>
<th>Average weight (kg)</th>
<th>Intestinal contents (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-7-91</td>
<td>1</td>
<td>2</td>
<td>Seine</td>
<td>0.22</td>
<td>1.92</td>
</tr>
<tr>
<td>9-18-91</td>
<td>2</td>
<td>7</td>
<td>Hook and line</td>
<td>0.59</td>
<td>2.27</td>
</tr>
<tr>
<td>10-8-91</td>
<td>3</td>
<td>4</td>
<td>Hook and line</td>
<td>ND ab</td>
<td>9.56</td>
</tr>
<tr>
<td>2-4-92</td>
<td>3</td>
<td>1</td>
<td>Seine, then held in cage for ca. 2 weeks</td>
<td>0.91</td>
<td>1.04</td>
</tr>
<tr>
<td>2-27-92</td>
<td>2</td>
<td>6</td>
<td>Hook and line</td>
<td>0.82</td>
<td>8.11</td>
</tr>
<tr>
<td>4-2-92</td>
<td>1</td>
<td>7</td>
<td>Seine, then held in cage for 2 d</td>
<td>0.59</td>
<td>3.71</td>
</tr>
<tr>
<td>6-16-92</td>
<td>3</td>
<td>4</td>
<td>Hook and line</td>
<td>0.59</td>
<td>11.14</td>
</tr>
<tr>
<td>6-30-92</td>
<td>2</td>
<td>7</td>
<td>Seine</td>
<td>0.50</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* ND = not determined.

were pooled into 100 ml 0.1% sterile peptone with 1% sodium hexametaphosphate as a wetting agent. The peritoneal cavity was opened aseptically, and the intestinal contents for each group emptied into a separate sterile stomacher bag. The bags were weighed and enough sterile 0.1% peptone added to make a 1:10 dilution. Samples were then homogenized for 1 min in a Stomacher 80 Lab Blender (Seward Medical, London). All serial dilutions were prepared in 0.1% sterile peptone.

Each group composite was analyzed for total plate counts using spread plates on plate count agar (Difco, Detroit, MI) and incubated aerobically and anaerobically at 35°C for 48 h, 22°C for 4 d, and 7°C for 7 d. Colonies from each set of aerobic plate counts with 25-250 colonies were numbered, and four colonies were selected from each set using a random number table to eliminate bias. Selected colonies were streaked to purity on trypticase soy broth agar (TSBA) prepared from 30 g Trypticase soy broth and 15 g agar (BBL, Cockeysville, MD).

Gram-negative bacteria were enumerated using spread plates on peptone bile amphotericin cycloheximide agar (8) incubated at 30°C for 48 h. Sporeformers were determined by heating 5 ml of the lowest dilutions in an 80°C water bath for 10 min. Samples were immediately cooled, spread plated onto plate count agar, and incubated aerobically and anaerobically at 35°C for 48 h.

The skin and gill samples were combined for the detection of specific pathogens. Enrichments and selective media were employed to detect the presence of Aeromonas spp. (32), Plesiomonas shigelloides (27), Salmonella spp. (26), Clostridium botulinum Type E (20), Clostridium perfringens (11), Staphylococcus aureus (6), and Listeria monocytogenes (24). Enrichments for Vibrio spp. (40) were performed for only the first three sampling rounds. Presumptive positive colonies were streaked to purity on TSBA plates for aerobes and brain heart infusion agar with blood plates (BHI BBL) (Carr Scarborough Microbiologicals, Stone Mountain, GA) for anaerobes.

A MIDI Microbial Identification System (Microbial ID, Inc., Newark, DE) was used to identify isolates based on fatty acid methyl ester (FAME) profiles. The MIDI system hardware and software, sample processing protocols, and chromatographic parameters were as described previously (35,41). Isolate profiles were compared to the Aerobe (TSBA) database or to the Anaerobe (BHI BBL) database to determine the most likely match. The Library Generation Software was used to conduct cluster analyses with dendrograms to separate the isolates into distinct groups based on their FAME profiles.

RESULTS

Skin
Aerobic incubation at 22°C resulted in the highest plate counts for the skin samples in all but one round (Fig. 1); aerobic counts at 22°C ranged from 87 to 3.1 x 10⁴ CFU/cm². Aerobic counts at 35 and 7°C averaged 94 and 82%, respectively, of the 22°C counts; the anaerobic counts averaged 87% of their aerobic counterparts. All sporeformer counts on the skin were less than 12 CFU/cm², except in samples taken on 10-8-91 which were 1,200 CFU/cm² under aerobic conditions and 690 CFU/cm² under anaerobic conditions.

Gills
Plate counts from the gills were below 60 CFU/cm² in two sampling rounds (Fig. 2); aerobic counts at 22°C ranged from less than 60 to 3.1 x 10⁴ CFU/cm². Anaerobic counts were 87% of the aerobic counts, and counts at 35 and 7°C were 95 and 97%, respectively, of the 22°C. All sporeformer counts from the gills were less than 60 CFU/cm².

Intestinal contents
The anaerobic counts at 22°C were higher than the other counts in all but one round, ranging from 4.7 x 10⁸ to 4.7 x 10⁹ CFU/g. The 35 and 7°C were 98.6 and 95% of those at 22°C, and the aerobic counts were 98% of the anaerobic counts (Fig. 3). All of the counts from Farm 3, 2-4-92, were greater than 10⁹ CFU/g. These fish contained only small amounts of mucoid material in their intestines, whereas the intestines of fish from the other sampling rounds contained digestfed feed. Sporeformer counts from all intestinal samples were less than 10⁹ CFU/g.

Water
The plate counts from the water samples ranged from 300 CFU/ml to greater than 10³ CFU/ml (Fig. 4). The highest total counts occurred at 22°C.

Bacterial isolates and identification
A total of 545 isolates were selected randomly from aerobic plate counts and 167 from the aerobic enrichments. The isolates were divided into groups based on their FAME profiles (Table 2). Only the random isolates were used in determining the frequency at which the different groups occurred on the fish.

Motile Aeromonas spp. were the most frequently isolated group of bacteria (Fig. 5) at all three temperatures and accounted for 27% of the total. The other predominant groups were coryneforms, (14%), Pseudomonas spp. (12%), Flavobacterium/Cytophaga/Sphingobacterium (FCS) complex (8%), Plesiomonas shigelloides (7%), Bacillus spp. (7%), and Enterobacteriaceae (6%).

More Aeromonas and Pseudomonas strains were isolated at 7 and 35°C than at 22°C, and Bacillus strains were more common at 22°C. The percentages of the Enterobacteriaceae and FCS bacteria isolated decreased with increasing incubation temperature; the reverse occurred with the coryneform group. P. shigelloides and Staphylococcus spp.
Figure 1. Plate counts from skin of aquacultured hybrid striped bass. Missing bars represent counts below 20 CFU/cm².

Figure 2. Plate counts from gills of aquacultured hybrid striped bass. Missing bars represent counts below 60 CFU/cm².

Figure 3. Plate counts from intestines of aquacultured hybrid striped bass.

Figure 4. Plate counts from water of aquaculture ponds. Missing bars represent counts below 10 CFU/ml.
TABLE 2. Species isolated from hybrid striped bass.

<table>
<thead>
<tr>
<th>Species</th>
<th>Percent of Isolated Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas spp.</td>
<td>18%</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>9%</td>
</tr>
<tr>
<td>Enterococci</td>
<td>8%</td>
</tr>
<tr>
<td>Carnobacterium</td>
<td>5%</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>4%</td>
</tr>
<tr>
<td>Microbacterium</td>
<td>3%</td>
</tr>
<tr>
<td>Micrococcus kristinae</td>
<td>2%</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>2%</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1%</td>
</tr>
<tr>
<td>Edwardsiella hoshinae</td>
<td>1%</td>
</tr>
<tr>
<td>Erwinia caratovora</td>
<td>1%</td>
</tr>
<tr>
<td>Erwinia chrysanthemi</td>
<td>1%</td>
</tr>
<tr>
<td>Erwinia herbicola</td>
<td>1%</td>
</tr>
<tr>
<td>Erwinia uredovora</td>
<td>1%</td>
</tr>
<tr>
<td>Enterobacter cancerogenus</td>
<td>1%</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>1%</td>
</tr>
<tr>
<td>Enterobacter taylorae</td>
<td>1%</td>
</tr>
<tr>
<td>Eschericia coli</td>
<td>1%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>1%</td>
</tr>
<tr>
<td>Klebsiella terrigena</td>
<td>1%</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>1%</td>
</tr>
<tr>
<td>Providencia alcalifaciens</td>
<td>1%</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>1%</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1%</td>
</tr>
<tr>
<td>Serratia odorifera</td>
<td>1%</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>1%</td>
</tr>
<tr>
<td>Vibrio cholera Inaba</td>
<td>1%</td>
</tr>
<tr>
<td>Vibrio cholera non 01</td>
<td>1%</td>
</tr>
<tr>
<td>Vibrio minicus</td>
<td>1%</td>
</tr>
<tr>
<td>Yersinia frederiksenii</td>
<td>1%</td>
</tr>
<tr>
<td>Yersinia pseudotuberculosis</td>
<td>1%</td>
</tr>
</tbody>
</table>

Figure 5. Microorganisms isolated randomly from aquacultured hybrid striped bass. *FCS = Flavobacterium/Cytophaga/Sphingobacterium group.

were isolated at 7°C; P. shigelloides was also not isolated from the sampling rounds in February and April. The Janthinobacterium lividum isolates were only isolated from the 2-4-92 sampling round, where they comprised 15% of the total.

Aeromonads accounted for 54% of the intestinal isolates, with P. shigelloides, Enterobacteriaceae, and Bacillus spp. comprising 18, 9, and 8%, respectively. The skin and gills were dominated by Aeromonas spp., Pseudo-

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hydrophila, P. shigelloides, and S. aureus were isolated from all farms. No Salmonella, Clostridium botulinum, or Clostridium perfringens were isolated. Though not selected for, Shigella dysenteriae was isolated from Farms 2 and 3. Yersinia pseudotuberculosis was isolated once from Farm 1, and Yersinia frederiksenii twice from Farm 2. Vibrio spp. were not isolated in the enrichments from the first three rounds.

Fish pathogens isolated included Carnobacterium piscicola, Enterobacter cloacae, Vibrio mimicus, Pseudomonas fluorescens, Aeromonas hydrophila, and species of Enterococcus and Streptococcus. Although a number of and monas fluorescens, Aeromonas hydrophila, were isolated. Though not selected Clostridium perfringens for the skin of live fish (23,38); unfortunately, most of the kidneys. The intensive aquaculture of fish in ponds or tanks the intestines and sometimes in the liver, spleen, heart, and humans consuming the fish. Detectable levels of bacteria, including Salmonella spp., have been found in the muscle tissue of aquacultured fish exposed to large populations of fecal bacteria (7,15,16).

Bacterial counts of 10² to 10⁵/cm² have been recorded for the skin of live fish (23,38); unfortunately, most of the data available are from the study of marine fishes. Horsley (17) reported counts of 10⁻¹ to 10³ bacteria per cm² on the skin of freshwater salmon in Scotland; pond-reared tilapia averaged 7.3 x 10² bacteria per cm² (1). In the present study, the highest skin count from aquacultured striped bass, 3.1 x 10⁴ CFU/cm², was within the ranges quoted in other studies. The gills also did not exceed 3.1 x 10⁴ CFU/cm², and the counts were frequently very low. Counts from the gills of freshwater hatchery-cultured salmonids in British Columbia averaged 3 x 10¹ organisms per g of gill tissue (43); the author estimated that the highest count of 9 x 10⁷/g of gill tissue represented 2.6 x 10⁵ organisms per cm².

MacFarlane et al. (25) reported counts of 3.1 x 10⁶ CFU/g for the intestines of wild striped bass in the Hudson River. Similar results were found in the present study, except in one round where the counts exceeded 10⁶ CFU/g. Bacterial counts from salmonid alimentary tract tissue plus its contents were as high as 10⁻¹⁰/g (44,49).

The predominance of Aeromonas in the intestines agrees with the findings of MacFarlane et al. (25) with wild striped bass from Long Island Sound. However, less than 2% of the intestinal flora in farm-raised striped bass were Pseudomonas spp., compared to 23% in the wild fish (25). P. shigelloides, which accounted for 18% of the intestinal isolates in the present study, was not isolated by MacFarlane et al. (25).

The gills and skin demonstrated greater variety in bacterial species than did the intestines. Both locations were dominated by Aeromonas, Pseudomonas, coryneforms, and FCS strains. Trust (43) found that the gills of freshwater salmonids were dominated by the same groups. Studies by Evelyn and McDermott (9) on Ontario trout indicated the predominance of Pseudomonas, Aeromonas, Micrococcus, and Lactobacillus species. Only six strains of Micrococcus and no Lactobacillus were isolated in the present study. The principal groups on the skin and gills of freshwater salmon were coryneforms, Moraxella, FCS, and Pseudomonas (17). Only one random isolate in the present study was identified as Moraxella. Moraxella-Acinetobacter, Micrococcus, Pseudomonas, Vibrio, and Flavobacterium were the primary groups isolated from pond-raised tilapia (1); Vibrios were isolated from only one round in this study. Other studies have cited the genera Alcaligenes, Moraxella, Achromobacter, and Alteromonas as composing large segments of the fish microflora (22,38), but less than 3% of the isolates in the present study were members of these genera.

Allen et al. (3) concluded that the microflora of freshwater fish farms have no adverse microbiological effects on the environment. Plate counts from a freshwater trout facility varied from 7 x 10⁴ to 1.65 x 10⁷/ml water (3), and water from tilapia ponds contained 2.2 x 10⁶ organisms per ml (1). Counts from this study fall within this range; it is likely that the environment surrounding the ponds has more effect on the pond microflora than the ponds have on the environmental microflora. The ponds are all adjacent to or very near to fields growing corn and soybeans; many of the isolates from this study are plant pathogens, e.g., Curtobacterium flaccumfaciens, Clavibacter michiganense, and the below grade pond from Farm 2, which is at greater risk of contamination from surface runoff than the above grade ponds, had the highest numbers of them.

Incubation at 22°C was the optimum temperature for isolating the greatest number and the widest variety of organisms from aquacultured striped bass, 7°C was the poorest temperature for growth, and 35°C counts were only slightly less than those at 22°C. This suggests that the pond populations were more mesophilic in nature than psychrophilic. Choice of incubation temperatures demonstrated a smaller effect on plate counts from the intestines than from the skin; this is likely due to the ability of the predominating aeromonads to grow well over a wide temperature range. Microflora differences also occur between freshwater and marine environments. Vibrios are more likely to be found in marine waters and aeromonads in freshwaters. This was demonstrated by the addition of salt to pond 4, which upset the dominance of the Aeromonas and allowed the outgrowth of Vibrio spp. and P. shigelloides.

Species of the family Vibrionaceae are widespread in aquatic environments and frequently compose part of the microflora of finfish. Motile aeromonads have been implicated as a cause of foodborne gastroenteritis in humans (29). The ability to grow from 0 to 45°C (46) and facultatively make them a concern in seafoods stored under refrigeration or modified atmospheres. A. hydrophila is readily destroyed by heat and irradiation and can be controlled by adjusting pH and NaCl concentrations (33). Therefore, Aeromonas spp. are more likely to be a problem in fresh striped bass than in a processed product. P. shigelloides may also be a cause of gastroenteritis (46); it does not grow below 8°C (18), which may explain why no
strains were isolated at 7°C or during the winter months. Since most of the *P. shigelloides* strains were isolated from the gut instead of the surface, fish that have been properly cleaned and stored below 8°C should pose little health risk. At least 11 species of the genus *Vibrio* are considered pathogenic to humans (10). They have frequently been found to comprise substantial portions of the intestinal flora of marine fish (2,22,25,31,36). Despite being widespread, outbreaks of cholera or diarrheal illness are rare and often result from the consumption of raw seafood. In this study, *Vibrio* spp. were isolated only from pond 4 after the addition of salt, and then at a low frequency. It is unlikely that vibrios will be a particular health hazard in striped bass from freshwater ponds.

*L. monocytogenes* has been isolated from a wide number of seafoods (12,30,48) and has been implicated in at least one outbreak of food poisoning from seafoods (21). The organism is more resistant to destruction by heat or irradiation than many other nonsporeformers, can grow facultatively, and can grow at temperatures as low as 1°C. Although the incidence of *Listeria* in this study was low, the potential severity of listeriosis and the hardness of this organism warrant cautious handling of the fish.

Although no *Salmonella*, *C. perfringens*, or *C. botulinum* were isolated in this study, it should not be assumed that these bacteria are absent in aquacultured striped bass. More extensive sampling may be necessary to detect low numbers of these organisms.

The numbers of bacteria isolated from hybrid striped bass reared in freshwater ponds do not exceed those reported in wild fish, and the incidence of organisms that may present a significant risk of foodborne illness is low. Therefore, hybrid striped bass raised in ponds do not present a greater hazard to consumers than do wild fish. Since aquacultured fish can be brought to market faster than wild fish, they probably pose a considerably lower microbiological health risk than wild fish.

**REFERENCES**


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