Growth of Inoculated Psychrotrophic Pathogens on Refrigerated Fillets of Aquacultured Rainbow Trout and Channel Catfish

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ABSTRACT

Aquacultured rainbow trout (Oncorhyncus mykiss) and channel catfish (Ictalurus punctatus) fillets were inoculated with the psychrotrophic pathogens Listeria monocytogenes and Aeromonas hydrophila: cell populations were monitored during refrigerated storage at 2 to 4°C. Fillets of both species were placed individually in sterile plastic bags and inoculated with cell suspensions (101.7 CFU/100 g of fish) of either A. hydrophila or L. monocytogenes or of both A. hydrophila and L. monocytogenes, for a total of three treatments for each species of fish. Each inoculum and fillet were mixed to ensure uniform distribution and then stored at 2 to 4°C. A. hydrophila, L. monocytogenes, and aerobic cell populations were determined on days 1, 3, 6, 8, 10, 13, and 15. Individually inoculated A. hydrophila and L. monocytogenes grew on catfish and trout fillets during the 15-day study. There was no inhibition of either pathogen by the natural flora on the fillets. Both psychrotrophic pathogens grew equally well in catfish and trout fillets inoculated with a combination of A. hydrophila and L. monocytogenes. In all three treatments, the counts of the psychrotrophic pathogens were lower than the aerobic plate counts. The growth of the psychrotrophic pathogens L. monocytogenes and/or A. hydrophila during refrigerated storage on aquacultured fish fillets could increase the food hazard risk, particularly where there is a possibility of cross-contamination with ready-to-eat food products.

Listeria monocytogenes and Aeromonas hydrophila are omnipresent psychrotrophic pathogens which have been isolated from a variety of fish and in growing waters (4, 8, 9, 10). Among the psychrotrophic pathogens, L. monocytogenes (3) is a recognized foodborne pathogen while A. hydrophila is an emerging pathogen. A. hydrophila has been identified as a cause of human gastroenteritis (12, 15) and an opportunistic pathogen (15, 16). Psychrotrophic pathogens are part of the natural microflora in aquacultured channel catfish (Ictalurus punctatus) and rainbow trout (Oncorhynchus mykiss) and hence have been isolated from fresh aquacultured products. Leung et al. (8) isolated L. monocytogenes and A. hydrophila from the viscera of channel catfish. DePaola et al. (2) also isolated A. hydrophila from market channel catfish. McAdams (9) isolated L. monocytogenes in both whole fish and fillets of aquacultured rainbow trout. Paniagua et al. (13) reported the isolation of motile Aeromonas spp. which were virulent to rainbow trout following intraperitoneal and intramuscular injections.

The sale of fish fillets and shellfish products containing psychrotrophic pathogens as their natural flora in supermarket display and other outlets presents several problems. The chief concerns are the growth of these psychrotrophic pathogens at refrigeration temperatures and the possible cross-contamination of the fresh fillets with cooked ready-to-eat products with the psychrotrophic pathogens during market handling or in the home refrigerator. Hence, the psychrotrophic pathogens are of concern as they may multiply to an undesirable level on refrigerated fresh fish during a normal refrigerated storage period. Foods appear to be a major vector of human infection with L. monocytogenes (15). Further, L. monocytogenes could proliferate on fish products (4, 7); aquacultured fish fillets are an excellent nutrient-rich substrate for growth of psychrotrophic pathogens.

Researchers have inoculated psychrotrophic pathogens on fish products and subsequently studied their proliferation (4, 7, 14). However, in reality more than one psychrotrophic pathogen may be present in a given food product. There is a paucity of data on the combined growth of important psychrotrophic pathogens such as L. monocytogenes and an emerging psychrotrophic pathogen such as A. hydrophila on fish fillets. Therefore, the objective of this study was to evaluate the individual and combined inoculation of A. hydrophila and L. monocytogenes on aquacultured channel catfish and rainbow trout fillets and to monitor their growth during refrigerated storage.

MATERIALS AND METHODS

Inoculum for spiking. The psychrotrophic pathogens Aeromonas hydrophila ATCC 7965 and Listeria monocytogenes ATCC 7644 were propagated in nutrient broth. About 100 μl of

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RESULTS AND DISCUSSION

FIGURE 1. Population of _A. hydrophila_ and _L. monocytogenes_ during 15 days of refrigerated (2 to 4°C) storage; counts were determined on day 1, 3, 6, 8, 10, 13, and 15 after inoculation.

Aquatricured fish fillets. Two aquacultured fish species were used in these studies. Fresh rainbow trout (_Onchorhyncus mykiss_) fillets were procured from an aquaculture farm in Virginia 1 day before the study began. Fillets were packaged in a polyethylene bag and transported on ice to the Virginia Tech campus. Upon arrival in the laboratory, 30 fillets were mixed by hand with a sterile spatula to achieve a uniform distribution of the indigenous microflora. Each fillet was trimmed to a weight of about 100 g (±2 g) and placed individually in a sterile 7 by 12 in stomacher bag (Seward, London, UK).

Individual quick frozen channel catfish (_Ictalurus punctatus_) fillets, about 125 g, were obtained from a local wholesale distributor and held frozen for a maximum of 15 days at −80°C. Two days prior to the initiation of the study, the fillets were allowed to thaw in a refrigerator (2 to 4°C). Thawed fillets were mixed with a sterile spatula to obtain a uniform distribution of microflora. Each fillet was trimmed to a weight of about 100 g (±2 g) and packaged in a sterile stomacher bag.

Quantitative analyses of psychrotrophic pathogens. Preliminary studies were performed to determine the base level of indigenous species of _Listeria_ and _Aeromonas_. Black _Listeria_–like colonies from samples before inoculation of rainbow trout and channel catfish fillets ranged from 0 to 27 and 0 to 51 CFU/100 g of fillet, respectively.

Farber (4) reported that the _L. monocytogenes_ count in cooked shrimp and cooked lobster was <10 CFU/g. McAdams (9) determined the frequency and number of several pathogens in whole fish and fillets of rainbow trout. _L. monocytogenes_ was identified in 20 to 90% of whole fish and 55 to 85% of fillets examined; however, the counts were very low in both whole fish (0.36 to 4.83 MPN/g) and fillets (0.73 to 3.98 MPN/g) (9). Leung et al. (8) observed that the visceral counts of _A. hydrophila_ and _Listeria_ spp. of pond-cultured catfish was 10^2.2 and 10^1.99 CFU/g (wet weight), respectively. Nedoluha and Westhoff (10) observed that 28% of the flora in aquacultured hybrid striped bass was due to _Aeromonas_ spp.

Since the indigenous background of _Listeria_–like and _Aeromonas_–like species were low, the catfish and trout fillets were inoculated with 10^2.7 CFU of each psychrotrophic pathogen per g. This inoculum level probably reduced and/or eliminated the background effect of the pathogens under investigation.

Growth of psychrotrophic pathogens on rainbow trout (_Onchorhyncus mykiss_). Figure 1 illustrates the geometric means of _A. hydrophila_ and aerobic plate counts on rainbow trout fillets during the 15-day storage period following inoculation. The aerobic plate counts increased significantly (P < 0.05) from 6.0 log CFU/100 g on day 1 to about 9.0 log CFU/100 g on day 15; the _A. hydrophila_ counts on each sampling day the fillets were also checked for odor; by these subjective analyses, the fillets were classified as either acceptable or spoiled.

Statistical design and analyses. Thirty-six fillets of each species were randomly divided into three whole plots (three treatments). The treatment design was split-plot with time (day of analysis) (17). The entire experiment was replicated three times and the data was analyzed as randomized complete blocks as the experimental design (18).

RESULTS AND DISCUSSION

Pathogenic quality of fillets. _Aeromonas_– and _Listeria_–like colonies from samples before inoculation of rainbow trout and channel catfish fillets ranged from 0 to 27 and 0 to 51 CFU/100 g of fillet, respectively.

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increased significantly ($P < 0.05$) from $4.5 \log \text{CFU/100 g}$ to $7.2 \log \text{CFU/100 g}$ during the same period. Inoculated *A. hydrophila* grew during the 15-day refrigerated storage in the presence of the indigenous microflora. However, after 10 to 13 days of storage, the fillets were considered to be unacceptable due to spoilage, a result of microbial decomposition as observed by subjective sensory analyses. Hazen et al. (6) observed that *A. hydrophila* can survive under a wide variety of environmental conditions. Abeyta and Wekell (1) showed that *A. hydrophila* is one of the components in the intestines of healthy fish and that the organism is widely distributed in nature. The presence of psychrotrophic pathogens such as *A. hydrophila* in fish products and their growth under refrigeration indicates that they could become a source of cross-contamination.

Figure 2 illustrates the geometric means for *L. monocytogenes* and aerobic plate counts following *L. monocytogenes* inoculation of rainbow trout fillets subsequently stored under refrigeration over a 15-day period. *L. monocytogenes* counts increased significantly ($P < 0.05$) by $2 \log \text{CFU/100 g}$; the aerobic plate counts increased significantly ($P < 0.05$) by approximately $3 \log \text{CFU/100 g}$ during refrigerated storage. Around 13 days the fillets emitted a putrid odor and were classified as unacceptable. Thus, *L. monocytogenes* proliferated on trout fillets in the presence of the natural microflora. The presence and proliferation of psychrotrophic pathogens such as *L. monocytogenes* could pose a problem if pathogens are transferred from the fish to the further processed food products (5).

Figure 3 shows geometric means of *A. hydrophila*, *L. monocytogenes*, and aerobic plate counts following simultaneous inoculation with *A. hydrophila* and *L. monocytogenes* on rainbow trout fillets. The aerobic plate counts increased significantly ($P < 0.05$) from $10^{6.3} \text{CFU/100 g}$ on day 1 to $10^{8.6} \text{CFU/100 g}$ on day 15. The *A. hydrophila* counts increased from $10^{4.7} \text{CFU/100 g}$ to $10^{9.9} \text{CFU/g}$ in 15 days; during the same time interval *L. monocytogenes* counts rose from $10^{5.2} \text{CFU/100 g}$ to $10^{10.8} \text{CFU/100 g}$. Both psychrotrophic pathogens could proliferate simultaneously on trout fillets in the presence of natural indigenous microflora. It did not appear that either of the inoculated psychrotrophic pathogens inhibited the growth of the other. At about 13 days the fillets were considered unacceptable due to decomposition (odor). The combined growth of the psychrotrophic bacterial pathogens during refrigerated storage compounds the problems associated with food safety.

**Channel catfish (Ictalurus punctatus).** The growth of individual and combined inoculated *A. hydrophila* and *L. monocytogenes* on channel catfish fillets stored at refrigeration temperature is shown in Figures 4 to 6. In catfish fillets inoculated with *A. hydrophila* (Fig. 4), the *A. hydrophila* and aerobic plate counts were similar to that of trout. The aerobic plate counts increased by 1,000-fold over a 15-day period, while the *A. hydrophila* count increased by $2.5 \log \text{CFU/100 g}$ during the same period. *A. hydrophila* also proliferated in the presence of the natural microflora. Ventura and Grizzle (20) observed that *A. hydrophila* gained access to the intestinal organs of catfish through the upper digestive tract and can result in motile *Aeromonas* septicemia. DePaola et al. (2) isolated oxytetracycline- and tetracycline-resistant *A. hydrophila* from cultured channel catfish. Leung et al. (8) isolated *A. hydrophila* from pond water and sediments as well as catfish viscera. Since these organisms are natural flora of catfish they would be naturally present and proliferate on aquacultured channel catfish. Leung et al. (7)
observed that when catfish samples were inoculated with *A. hydrophila* the population grew rapidly and increased from 4.0 log CFU/cm² on day 1 to 6.5 to 7.0 log CFU/cm² on day 16. Additionally, *A. hydrophila* grew well under aerobic conditions (7, 11). The conditions in this study were aerobic, which may have facilitated *A. hydrophila* growth.

The geometric means of *L. monocytogenes* and aerobic plate counts following inoculation of catfish fillets are shown in Fig. 5. *L. monocytogenes* counts increased significantly (P < 0.05) from 10⁵.1 CFU/100 g on day 1 to 10⁹.9 CFU/100 g on day 15, while the aerobic plate counts increased significantly (P < 0.05) from 10⁶.1 CFU/100 g to 10⁸.9 CFU/100 g during the same period. Inoculated *L. monocytogenes* proliferated on catfish fillets in the presence of the inherent catfish microflora, just as did the growth of *L. monocytogenes* on rainbow trout fillets observed in this study. The catfish fillet samples emitted a bad odor by about the 10th day of storage. Leung et al. (7) observed that the *L. monocytogenes* population increased slowly by approximately 1 to 1.5 log cycles over the first 12 days when *L. monocytogenes* was inoculated on channel catfish. However, their count decreased by about 1.5 log cycles on the 16th d. The differences between this and the study by Leung et al. (7) were due to the microbial species, packaging conditions and microbial enumerations.

When *L. monocytogenes* and *A. hydrophila* were concurrently inoculated on catfish fillets their numbers increased (Fig. 6). The *A. hydrophila* count increased from 4.6 log CFU/100 g to 6.5 log CFU/100 g over a 15-day interval. During the same period, the counts of *L. monocytogenes* and aerobic plate counts increased by about 2.0 and 2.5 log CFU/100 g respectively. When *L. monocytogenes* and *A. hydrophila* were inoculated simultaneously on the catfish fillets, both microorganisms produced similar growth. These results are similar to those obtained for trout fillets when a combination of the psychrotrophic pathogens were inoculated.

It is evident that both individually and simultaneously inoculated psychrotrophic pathogens *L. monocytogenes* and *A. hydrophila* can grow on aquacultured catfish and rainbow trout fillets at refrigeration temperatures (2 to 4°C). However, the inherent natural flora associated with the fillets was responsible for spoilage and outgrew the inoculated psychrotrophic pathogens.

The presence of psychrotrophic pathogens in fresh aquacultured processed fish in low numbers in itself is not a problem, because these products are thermally processed, thereby reducing the risk to the consumers. The chief concern is the problem of cross-contamination with cooked ready-to-eat processed products. If the pathogens outgrow the normal flora then they may result in a food safety problem. However, since fresh catfish and rainbow trout fillets would primarily undergo spoilage prior to pathogen proliferation the issue is of food quality and not food safety.

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