Common Bacterial Species Associated with Fish

Sabeehah Rehman and Ruqeya Nazir*

Centre of Research for Development, University of Kashmir, Srinagar-190006, J & K, India
*Corresponding author: ruqeya.ku@gmail.com

Abstract
Fish are one of the most beneficial and nutritional resources of human beings. Fish quality and the microbes associated with fish are directly linked to human health. Bacteria invade almost all organs of the fish including skin, gills and gastrointestinal tract etc. Numerous bacteria taxa have been found to be associated with most of the fish. Both marine and fresh water fish are affected by different bacteria species. However, bacterial association also depends on the surrounding water quality as well. Commonly associated bacteria with fish include *Aeromonas spp., Bacillus spp., Pseudomonas spp. Vibrio spp. Flavobacterium,* etc.
Keywords: fish, microbes, bacteria, association

Introduction
With the development of commercial aquaculture, it has become apparent that diseases can be a significant limiting factor. Major bacterial pathogens of fish include the Gram-negative species, *Aeromonas salmonicida*, *Vibrio anguillarum*, *Vibrio salmonicida* and *Yersinia ruckeri*, the etiological agents of furunculosis, vibriosis, cold-water vibriosis and red mouth disease respectively. In addition, *Aeromonas hydrophila* may cause infections in fish and in Aquaculture Research, generally associated with small surface lesions, sloughing of scales, local haemorrhage and septicaemia. All these diseases are common worldwide and produce considerable economic losses during intensive aquaculture of trout and salmon (Austin and Austin, 1999). The results of numerous studies indicate that fish possess bacterial populations on or in their skin, gills, digestive tract, and light-emitting organs. In addition, the internal organs (kidney, liver and spleen) of healthy fish may contain bacteria, but there is debate about whether or not muscle is actually sterile. Before an infection can be established, pathogens must penetrate the primary barrier. The three major routes of infection are through skin, gills (Birkbeck and Ringo, 2005) and Gastrointestinal (GI) tract (Sakai, 1979; Rose *et al.,* 1989)

Diversity of bacteria in fish
Surface microflora
The bacteria from the surface of freshwater fish have been reported to include *Acinetobacter johnsonii* (Gonzalez *et al.*, 2000) *Aeromonads* (notably *Aeromonas hydrophila, A. bestiarum, A. caviae, A. jandaei, A. schubertii,* and *A. veronii biovar sobria* (Gonzalez *et al.*, 2001), *Alcaligenes piechaudii, Enterobacter aerogenes, Escherichia coli, Flavobacterium* (Zmyslowska *et al.*, 2001)*, Flexibacter* spp., *Micrococcus luteus*, Moraxella spp., *Pseudomonas fluorescens*, *psychrobacters* (Gonzalez *et al.,* 2000), and *Vibrio fluvialis* (Diler *et al.*, 2000). To some extent, the presence of aeromonads reflected whether or not the water in which the fish occurred was polluted or cleaned (Gonzalez *et al.,* 2001). Bacteria, typical of those in seawater, have been recovered from the surface of marine fish and include *Acinetobacter calcoaceticus, Alcaligenes faecalis, Bacillus cereus, B. firmus, Caulobacter, coryneforms, Cytophaga/Flexibacter, E. coli, Hyphomicrobium vulgare, Lucibacterium (Vibrio) harveyi, Photobacterium angustum, P. logei, Prosthecomicrobium, Pseudomonas fluorescens, P. marina,* and *Vibrio spp.* (Montes *et al.,* 1999). As a result of a detailed numerical taxonomic study of Gram-negative, oxidase-

positive bacteria recovered from sharks, the dominance of vibrios was noted, with representatives including *V. harveyi* and *V. alginolyticus*. Other groups included *Aeromonas, Photobacterium (including P. damselae* and *P. damselae sub sp. piscicida), Alteromonas, Plesiomonas shigelloides, Moraxella,* and *Neisseria* (Grimes *et al.,* 1993).

Gill microflora
Yellow-pigmented, Gram-negative rods, especially *Cytophaga spp.* dominate on gills (Trust, 1975). Aeromonads, coryneforms, enterobacteria, Gram-positive cocci, pseudomonads, and vibrios have also been recovered from the gills of healthy juvenile rainbow trout (Nieto *et al.*, 1984). Gills of fish accommodate *Achromobacter*, *Alcaligenes*, *Bacillus, Flavobacterium*, and *Micrococcus* (Shewan, 1961) and yellow-pigmented bacteria, loosely associated with Chryseo bacterium- Flavobacterium - Flexibacter- Cytophaga (Mudarris and Austin, 1988).

Microflora in the digestive tract
Studies on the microflora of the digestive tract have led the way in the use of culture-independent approaches (Huber *et al.*, 2004). However, the bulk of the historical data stems from culturing methods, which will be discussed first. Ringo *et al.* (1995) have written an excellent review on the topic. Initially in the sac fry, only a few taxa (coryneforms and pseudomonas) occur within the digestive tract (Yoshimizu *et al.*, 1980). It is likely that some bacteria become ingested at the yolk-sac stage, leading to the establishment of an initial intestinal microflora (Hansen and Olafsen, 1999). In addition, it has been reported that bacterial colonisation of the digestive tract of turbot larvae coincided with the start of feeding, when the microflora was dominated by Aeromonas and Vibrio (Munro *et al.*, 1994). In an investigation of the intestinal microflora of larval sea bream (*Dicentrarchus labrax*) and sea bass (*Sparus aurata*), it was observed that when the larvae were fed with rotifers, there was a high incidence of *V. anguillarum, V. tubiashii,* and *nonvibrio*. However, feeding with Artemia led to the recovery of mostly *V. alginolyticus, V. proteolyticus, V. harveyi, and V. natriegens*. It was concluded from these experiments that the fluctuations in the dominant components of the microflora reflected the bacteria in the live feed. Indeed, the dominance of vibrios was not recorded until the end of the larval stage (Grisez *et al.*, 1997). The comparative lack of diversity in larvae continues into older fish, and it has been suggested that the flora may be subjected to as-yet undescribed selective effects leading to a restricted number of taxa being present (Liston, 1957).

A comparatively wide range of taxa have been associated with the digestive tract of adult freshwater fish and include *Mycoplasma* (Holben *et al.*, 2002) *Acinetobacter*, *Enterobacter, Escherichia, Klebsiella, Proteus, Serratia, Aeromonas, Clostridium and Fusobacterium* (Trust and Sparrow,1974). Isolates have been identified by microplate hybridization as *A. caviae, A. hydrophila, A. jandaei, A. sobria,* and *A. veronii* (Sugita, *et al.*, 1994). *Alcaligenes, Eikenella* (Lee and Lee, 1995), *Bacteroides*(Kamei, *et al.*, 1985), *Citrobacter freundii* (Apun *et al.*, 1999), *Hafnia alvei* (Ugajin *et al.*, 1979), Cytophaga/Flexibacter (Nieto *et al.*, 1984), *Bacillus, Listeria, Propionibacterium, Staphylococcus* (Apun *et al.,* 1999), *Moraxella* (Diler and Diler, 1998), and *Pseudomonas*. In one study involving pike perch, it was concluded that *Moraxella* and *Staphylococcus* were unique to the habitat when compared with the digestive tract of other fish species (Diler and Diler, 1989). Modern phenetic and molecular-based studies, including 16S rRNA sequencing have indicated variability in the intestinal microflora of salmonids, notably rainbow trout and Atlantic salmon reflecting the fish farm of origin (Huber *et al.*, 2004), with analyses revealing the dominance of the gamma subclass (Spanggaard *et al.*, 2000) and beta subclass of *Proteobacteria*, and Gram-positive bacteria with a low G + C-content of the DNA (*Carnobacterium*). The approaches have permitted the recognition of potentially new taxa. For example, a 16S rRNA gene sequence with similarity to *Anaerofilum pentosovorans* has been detected. In one detailed study, 41 culturable microbial phylotypes, and 39 sequences from 16S rRNA and 2 from 18S rRNA genes were retrieved from the digestive and

intestinal mucus of rainbow trout and equated largely with *Aeromonadaceae, Enterobacteriaceae (i.e., Buttiauxella, Enterobacter, Hafnia, Pantoea, Plesiomonas,* and *Proteus*) and Pseudomonadaceae representatives. Intestinal contents contained *Arthrobacter, Bacillus, Carnobacterium, Exiguobacterium, Flavobacterium, Kokuria, Microbacterium, Micrococcus, Rhodococcus, Sporocytophaga,* and *Ultramicrobacterium*. Genomic DNA isolated from intestinal contents and mucus was used to generate 104 random clones, which were mostly affiliated with *Proteobacteria* (>70% of the total). Twelve sequences were retrieved from denaturing gradient gel electrophoresis analysis of the digestive tract or rainbow trout, and dominant bands were mostly related to *Clostridium* (Kim *et al.*, 2007). One of the outcomes of the study was the realization that *Capnocytophaga, Cetobacterium, Erwinia, Porphyromonas, Prevotella, Rahnella, Ralstonia, Serratia,* and *Veillonella* were recognised as occurring for the first time as culturable components of the microflora in the digestive tract of freshwater fish. Using a parallel approach, the digestive tract of wild and farmed salmon from Norway and Scotland were found to be populated with *Acinetobacter junii* and a novel *Mycoplasma* phylotype, the latter of which comprised almost all, i.e., ~96%, of the microflora of the distal intestine of wild salmon Holben *et al.,* 2002). The digestive tract of adult marine fish has been reported to contain *Aeromona, Alcaligenes, Alteromonas, Carnobacterium* (Ringo *et al.*, 2001), *Flavobacterium, Micrococcus, Photobacterium, Pseudomonas, Staphylococcus, and Vibrio*, including *V. iliopiscarius*. Terminal restriction fragment length polymorphism data point to a greater diversity in the posterior compared to the anterior gut in large herbivorous fish, i.e., *Kyphosus sydneyanus* (Moran *et al.*, 2005). Special groups, such as large (gigantobacteria) symbiotic bacteria, have been observed in the digestive tract of surgeonfish from the Red Sea and Indo-Pacific Region (Fishelson, 1999). Also, using a specific nested polymerase chain reaction, methanogens have been detected in the digestive tract and faeces of flounder (*Platichthys flesus*) from the North Sea (van der Maarel, 1999). Indeed, in this study, 16S rDNA sequences revealed 97.6–99.5% similarity to the archaea representative *Methanococcoides methylutens*. Lactic acid bacteria, notably *Carnobacteria*, are common associated with fish, particularly in the digestive tract (Ringo *et al.,* 1998) with investigations highlighting the presence of *Lactococcus* notably *L. lactis* and *L. raffinolactis* (Hagi *et al.,* 2004). To date, studies have emphasised the taxonomy of the organisms, highlighting the presence of *Carnobacterium particularly C. piscicola and C. piscicola* like bacteria (Ringo *et al.*, 2000), and their role as putative probiotics for use in aquaculture. Other lactic-acid bacteria present in the epithelial mucosa have been equated with *Lactobacillus plantarum, Leuconostoc mesenteroides,* and *Streptococcus spp.* In a separate investigation, *Lactobacillus, Enterococcus durans, Lactococcus, Vagococcus, C. divergens,* and *C. piscicola* were recovered from freshwater fish, notably brown trout (Salmo trutta), and characterized phenotypically by numerical analyses (Gonzalez *et al.*, 2000). A previously undescribed species, *C. inhibens*, was recovered from the intestine of Atlantic salmon, and demonstrated antibacterial activity against fish pathogens, notably *Aeromonas salmonicida* and *Vibrio anguillarum* (Joborn *et al.*, 1999).

Diets
Aeromonads, Bacillus, Pseudomonads, and Staphylococcus dominate in diets (Kitao and Aoki, 1976).

Eggs
Healthy eggs are populated by Cytophaga/Flavobacterium and, to a lesser extent, *Pseudomonas* (Bell *et al.*, 1971), reflecting the organisms present in water (Hansen and Olafsen, 1999).

Internal organs
The liver and kidney of healthy turbot have been found to be populated by mostly *Pseudomonas and Vibrio,* including *V. fischeri, V. harveyi, V. pelagius,* and *V. splendidus*. Similarly, *Shewanella spp*. has been recovered

from the internal organs (Decostere *et al.*, 1996). The reasons for the presence of some of these bacteria are unclear. Moreover, it is speculative whether or not the fish are at the earliest stage of an infection cycle.

Biofilms – microbial shelters

In most natural environments, microbes attach to surfaces, multiply and form biofilms which provides enhanced resistance to external disturbances. In this state the biofilm associated cells are 5 more resistant to many toxic substances such as antibiotics, chlorine and detergents (Watnick and Kolter, 2000). The formation of biofilms is usually depicted as a series of discrete stages in life cycle which begins when planktonic cells contact surfaces, either randomly or by chemical attractants. The next steps involve irreversible attachment when cells have multiplied and have started to secrete extracellular polymeric substances such as polysaccharides, proteins and DNA. After that the biofilm maturates and disperses (Simoes *et al.*, 2009). Bacteria living in biofilms are believed to communicate by chemical signalling although orchestrated behaviour of the community is a matter of dispute. A change in gene expression when cells go from planktonic state to biofilms is unquestionable and is the underlying cause of different cell behaviour that characterizes biofilms (Davey and O'Toole, 2000). Although no single mechanism is responsible, many species use quorum sensing to modulate surface attachment, motility, extracellular polymeric production and dispersal (Dunne, 2002). Secreted polymers are defining feature of biofilms but the functions of them are not yet entirely clear. They promote surface attachment and provide structural support but also offer protection from external threats or help secreting strains to grow toward nutrient rich locations (Nadell, 2009, Xavier and Foster 2007). A mature biofilms is usually composed of channels and cavities to allow the exchange of nutrients and waste. Biofilms are not restricted to natural habitats as food processing facilities are ideal environment for biofilm formation where nutrient rich liquid constantly or periodically covers the surfaces. This can cause problem to the production if proper hygienic preventive measures are not performed. Undesirable bacteria such as spoilers (*Pseudomonas spp*.) and pathogens (*Listeria monocytogenes*) have been shown to form biofilms in food processing plants and if the biofilm grows to mature state the threat of persistent contamination of these bacteria in the food is apparent (Bagge-Ravn *et al.*, 2003).

Conclusion

Fish like any other taxa remain associated with numerous bacteria species. The bacteria invade most of the parts of the fish. However association of bacteria with fish also depends on the quality of water in which the fish dwells. Characterization of microbial communities in processing facilities can contribute to better design of hygienic programs and processing equipment that minimizes the risk of accumulation of undesirable microbes in processing surfaces such as spoilage organisms and pathogens.

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