[3] Diseases Caused By Bacterial Pathogens In Inland Water

Summary

Bacterial diseases cause huge damages in fish farms worldwide, and numerous bacterial pathogens from inland and saline waters have been identified and studied for their characterization, diagnosis, prevention and control. In this chapter, eight important fish diseases *viz.* 1) streptococcosis (inland water), 2) furunculosis, 3) bacterial gill disease, 4) columnaris disease, 5) bacterial cold-water disease, 6) red spot disease, 7) edwardsiellosis (*Edwardsiella ictaluri*), and 8) motile aeromonads from inland water were included covering the topics such as characteristics of disease agent, and pathogenesis, histopathological interest, diagnostic method, chemotherapy and disease control.

1. INLAND WATER STREPTOCOCCOSIS

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1.1. Synopsis

Streptococcosis caused by the genera *Streptococcus* and *Lactococcus*, occurs in cultured and wild fish in freshwater, brackish water, and seawater environments due to the worldwide development of intensive fish farming practices. The genera *Streptococcus* and *Lactococcus* are facultative anaerobic, catalase-negative, and morphologically Gram-positive cocci. Historically, hemolysis on blood agar and Lancefield serological grouping have been used to identify and classify pathogenic *Streptococcus* spp. Fish pathogenic *Streptococcus* spp. and *Lactococcus* spp. are also classified into α, β, and γ (non-hemolysis) hemolysis types and Lancefield groups B, C, and non-typable.

Inland freshwater streptococcosis in cultured fish is caused by several bacterial pathogens, including *L. garvieae*, *L. piscium*, *S. iniae*, *Vagococcus salmoninarum*, and Lancefield serological group B *S. agalactiae* (GBS) and group C *S. dysgalctiae*. *L. garvieae*, *S. iniae*, and *S. agalactiae*, and *S. dysgalactiae* cause serious diseases in freshwater fish and in cultured and wild saltwater fish. In particular, a mass mortality of wild mullet occurred in Kuwait Bay due to β-hemolytic *S. agalactiae* infection (Evans *et al*., 2002) (Figure 1.1). Although *S. agalactiae* causes diseases in marine fish, this section focuses on Lancefield serological group B *S. agalactiae* (GBS), *L. piscium*, and *V. salmoninarum* as causal agents of streptococcosis in freshwater fish. *L. garvieae, S. iniae, and S. dysgalactiae* also cause diseases in salmonids, sweetfish (*Plecoglossus altivelis*), or tilapia in freshwater environments. These pathogens will be described in the section on bacterial pathogens in saltwater streptococcosis.

1.2. Introduction

Inland water streptococcosis occurs in freshwater fish species, mainly tilapia and salmonids. *S. agalactiae* causes diseases in warm water species including tilapia. *S. agalactiae* infections occur in cultured and wild fish species in both marine and freshwater environments in many countries including Australia, Brazil, Kuwait, Israel, USA, and Thailand. Streptococcosis in salmonids under low water temperature conditions is caused by *V. salmoninarum* and *L. piscium* infections. This section also introduces these pathogens.

Figure 1.1. *Streptococcus agalactiae* infection in wild fish in the Kuwait bay (Photo courtesy of Prof. M. Endo, Tokyo University of Marine Science and Technology)

1.3. *S. agalactiae* (= *S. difficilis*), a Disease Agent

S. agalactiae belongs to the Lancefield group B serotype. In general, Lancefield group B streptococci are synonymous name as *S. agalactiae*. Previously identified as non-hemolytic *S. difficilis* (=*S. difficile*. Eldar *et al*., 1994) and mainly isolated from tilapia, it was originally described as a non-typable Lancefield serological group. Later, *S. difficilis* (=*S. difficile*) was found to belong to Lancefield group B and capsular polysaccharide antigen type Ib (Vandamme *et al*., 1997), and this bacterial classification was proposed as a later synonym of *S. agalactiae* (Kawamura *et al*., 2005)

S. agalactiae (= *S. difficilis*) infections are found in many freshwater and marine fish species (Evans *et al*., 2002; Mian *et al*.; 2009; Geng *et al*., 2012). In particular, *S. agalactiae* exhibited high virulence during infection trials in Nile tilapia *Oreochromis niloticus*, which is an important cultured freshwater fish (Mian *et al*., 2009). *S. agalactiae* isolates from fish were positive for hippurate hydrolysis and the Voges-Proskauer reaction. The isolates were negative for the pyrolidonyl arylamidase reaction and hydrolysis of urea and starch. Acid was produced from ribose, but not from sorbitol, mannose, and xylulose. Hemolytic (beta) and non-hemolytic strains were isolated from fish (Evans *et al*., 2008). Streptococcosis in tilapia aquaculture is mainly caused by *S. agalactiae* and *S. iniae.* These strains are distributed in several countries. Table 5.1 in the section on *S. iniae Diseases caused by Bacterial Pathogens in Saltwater*: Saltwater Streptococcosis) shows the different bacteriological characteristics between *S. agalactiae* and *S. iniae*.

1.4. Diagnostic Methods

1.4.1. Serological Classification

S. agalactiae (= *S. difficilis*) from fish possess a Lancefield serological group B antigen and capsular serotype Ib antigen (Vandamme *et al*., 1997). Evans *et al* (2008) reported that fish isolates (serotype previously unreported in group B *Streptococcus*) from several countries (Kuwait, Brazil, Israel and USA) were typed as capsular serotype Ia. The ability

of BioStar STREP B Optical ImmunoAssay (STREP B OIA, a BioStar^{R}OIA^{R}Strep B assay kit; BioStar Incorporation, Louisville, CO, USA) to identify GBS isolated from aquatic animals was evaluated and found to be useful for identifying GBS strains cultured or directly collected from clinically infected fish (Evans *et al*., 2010).

1.4.2. Clinical Signs and Pathogenicity

The virulence of *S. agalactiae* isolated from fish, bovine, and humans was investigated in Nile tilapia. Some strains isolated from fish, bovine, and humans caused meningoencephalitis in the fish (Pereira *et al*., 2010). Fish infected with GBS may swim erratically and spirally, and exhibits darkened coloration. Exophthalmia and corneal opacity are typical clinical signs of infected fish. Periocular and intraocular hemorrhage was also observed in some infected fish (Bowater *et al*., 2012) (Figure 1.2). In experimental infections, septicemia with severe mononuclear infiltration was found in the meninges, epicardium, and eye (Filho *et al*., 2009).

Figure 1.2. Affected fish showing intraocular hemorrhages (Photo courtesy of Prof. M. Endo, Tokyo University of Marine Science and Technology)

1.4.3. PCR for Identification

Several PCR assays targeting species-specific regions of *S. agalactiae* 16S rRNA (Shome *et al*., 2011) for multiplex PCR assay and 23S rRNA have been developed (Kawata *et al*., 2004).

1.4.4. Molecular Classification

A high similarity was observed in whole genome DNA-DNA hybridization between *S. agalactiae* and *S. difficilis* (Kawamura *et al*., 2005). The genetic relatedness of fish, dolphin, human, and bovine GBS strains isolated from different geographical regions was examined using multilocus sequence typing (Evans *et al*., 2008). Phylogenetic trees of house-keeping genes (*gyrB, sodA, gyrA,* and *parC*) revealed that fish isolates of *S. difficilis* and *S. agalactiae* composed one cluster in which other pyrogenic *Streptococcus* species isolated from animals were not included (Kawamura *et al*., 2005).

1.4.5. Genome Analysis

Recently, *S. agalactiae* STIR-CD-17 genome was submitted to NCBI. The non-hemolytic strain was isolated from a moribund fish during a disease outbreak in farmed tilapia (*Oreochromis* sp.) in Honduras in 2008. The draft genome sequence of STIR-CD-17 has been deposited in GenBank under the accession number ALXB00000000 (Delannoy *et al*., 2012).

1.5. Control

1.5.1. Vaccine

An oil-adjuvant vaccine against *S. agalactiae* serotype II (Ib) was commercialized for tilapia and other susceptible species in Brazil and Indonesia (http://www.merk-animal-health.com/news/). A concentrated extracellular product (ECP) vaccine for *S. agalactiae* was developed for 30-g tilapia to induce antibody-mediated immunity through intraperitoneal and bath immersion administration (Evans *et al*., 2004).

1.5.2. Vagococcus Salmoninarum

V. salmoninarum infection is an emerging disease in European trout farms (Daly, 1999). The genus *Vagococcus* is motile, similar to the genus *Lactococcus*, and a new species has been proposed as *V. fluvialis* (Collins *et al*., 1989). Atypical lactobacilli isolated from diseased salmonid fish were identified as *Vagococcus* spp. Analysis of 16S rRNA sequence data clearly indicated that atypical lactobacilli isolated from fish were phylogenetically closer to the genera *Enterococcus* and *Vagococcus* than to the genus *Lactobacillus*. Atypical fish lactobacilli strains were found to belong to the genus *Vagococcus* and a new species different from *V. fluvialis* was identified as *V. salmoninarum* (Wallbanks *et al*., 1990). Wallbanks *et al*. (1990) reported a detailed description of *V. salmoninarum* isolated in USA. Schmidtke and Carson (1994) also reported on *V. salmoninarum* isolated in Tasmania, Australia, and Norway, in addition of new finding data to the report by Wallbanks *et al.* (1990).

V. salmoninarum grows at 5°C and 30°C, but not at 40°C; it produces acid from amygdalin, cellobiose, fructose, glucose, maltose, mannose, salicin, starch, sucrose, and trehalose; it produces H_2S and its G+C content was between 36.0 and 36.5 mol% (Wallbanks *et al*., 1990). Salmonid strains exhibited α-hemolysis on sheep's blood agar and grew at pH 9.6, 10 and in 4% NaCl w/v, but not 6.5% NaCl. Lancefield serological group D and N antigens were not detected. *V. salmoninarum* could be differentiated from similar fish pathogens including *L. garvieae* (=*Enterococcus seriolicida*) and *L. piscium* (Schmidtke and Carson,1994).

1.5.3. Pathogenicity

Vagococcosis occurs at lower water temperatures (10°C−12°C) compared to other streptococcosis (Michel *et al*., 1997). *V. salmoninarum* causes chronic infections and drug treatments were ineffective in rainbow trout. Hyperemia and hemorrhage occurred in

cardiovascular lesions in the gills and viscera of infected rainbow trout (Michel *et al*., 1997).

1.5.4. L. Piscium

A lactic acid bacterium of uncertain taxonomic position isolated from diseased salmonid fish was identified as *L. piscium* (Williams *et al*., 1990). Chemical and molecular taxonomic studies such as fatty acid analysis, DNA base composition, and 16S rRNA sequencing were performed on a typical unknown lactic acid bacterium isolated from diseased salmonid fish and related bacteria including *Vagococcus* spp. (*V. fluvialis* and *V. salmoninarum*). Based on these results and detailed bacteriological charcteristics, these unknown bacteria were proposed to comprise a new species, *L. piscium* sp. nov. (Williams *et al*., 1990). Williams *et al*. (1990) reported *L. piscium* as follows: (1) its cell shape was either short rod-like or ovoid; (2) it could grow at 5°C and 30°C, but not at 40°C; (3) it could not produce H_2S; and (4) its G+C content was 38.5 mol%, as determined by melting temperature.

1.6. Recent Topics

1.6.1. Emerging Streptococcosis

Mortalities of channel catfish (*Ictalurus punctatus*) brood stock caused by unidentified streptococcal infections have been observed at several aquaculture sites in the Mississippi Delta. The main causes of mortality were arthritis, osteolysis, myosis, and spinal meningitis. DNA-DNA hybridization, 16S rRNA analysis, and other biochemical tests revealed the causative agents belonged to the genus *Streptococcus*, and a new species, *S. ictaluri*, was proposed by Shewmaker *et al*. (2007).

2. FURUNCULOSIS

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2.1. Synopsis

Furunculosis, caused by the Gram-negative, non-motile, fermentative, rod-shaped bacterium, *Aeromonas salmonicida*, is one of the most serious infectious diseases of wild and farmed salmonids. The disease was first described 120 years ago from trout hatchery in Germany. Since that time, the disease and its etiological agent have been found in most salmonid hatcheries and many wild populations throughout much of the world. The pathogen can be readily isolated from kidney tissues of dead or moribund fishes using commercial media. Oral administration of antimicrobial compounds is useful for control. For prevention of this disease, vaccines are used. In spite of considerable knowledge of chemotherapy and control, furunculosis continues to be a major problem in hatcheries.

2.2. Introduction

Furunculosis, caused by the bacterial pathogen, *Aeromonas salmonicida*, is a globally important disease affecting wild and cultured stocks of salmonids and other fish species. Furunculosis was first described in the 18th century in a brown trout hatchery in Bavaria,