[3] Diseases Caused By Bacterial Pathogens In Inland Water

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

Tetsuichi Nomura

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,

Germany where the manifestations of the disease included furuncule-like swellings and, at a later stage, ulcerative lesions on infected trout (Bernoth, 1997). The common name of the disease is derived from the presence of “blisters” or furuncles on the surface of chronically infected salmonids. After the initial description, numerous reports in the literature described the epizootiology and control of the disease (Schachte, 2002; Toranzo et al, 2005; Cipriano and Bullock, 2001) and the ability of both “typical” and “atypical” strains of the bacterium to cause disease (Wiklund and Dalsgaard, 1998). In spite of considerable knowledge of chemotherapy and control, furunculosis continues to be a major problem in hatcheries.

2.3. Host Range

Furunculosis occurs in many species of salmonid fish in freshwater and seawater, but the level of susceptibility is variable (Bernoth, 1997; Cipriano and Bullock, 2001). For example, among salmonids, susceptibility to infection is reported to be low in rainbow trout, while brook trout, brown trout and many other salmon species appear to have a high susceptibility. In addition, susceptibility may vary within the same fish species raised from different genetic lines, age or with different histories of exposure to the various subspecies of *A. salmonicida*. In Atlantic salmon farms, a high percentage of the fish losses are attributable to furunculosis. Spawning and smolting fish are prime victims of furunculosis due to their compromised immune status according to Department of Agriculture, Fisheries and Forestry (2009).

2.4. Disease Agent

The most important aetiological agent of furunculosis in salmonids is *Aeromonas salmonicida* subsp. *salmonicida*, which is commonly known as the “typical” strain of *A. salmonicida* and is probably the most commonly encountered bacterial pathogen in salmonids.

Characteristics: The pathogen was first described by Griffin et al. (Griffin et al, 1953). Since that time a number of subspecies of *A. salmonicida* have been recognized, although the taxonomy of the species is far from settled. Although Bergey's Manual of Systematic Bacteriology recognizes five subspecies of *A. salmonicida*: *salmonicida*, *achromogenes*, *masoucida*, *smithia*, and *pectinolytica*, many laboratories currently classify *A. salmonicida* subsp. *salmonicida* as "typical" and any isolate deviating phenotypically as "atypical". *A. salmonicida*’s ability to infect a variety of hosts, multiply, and adapt, make it a formidable pathogen (Martin-Carnahan and Joseph, 2005). *A. salmonicida subsp. salmonicida* comprises non motile, fermentative, gram-negative rods, typically 1µm x 2µm, cytochrome oxidase positive which produce a brown water-soluble pigment on tryptone-containing agar, do not grow at 37˚C, and produce catalase and oxidase. Other subspecies of *A. salmonicida* do not produce this brown pigment. Some strains of *A. salmonicida* may be cytochrome oxidase negative, a result that is inconsistent for this species. The history of the organisms reveals a plethora of synonyms including: *Bacillus devorans*, *Bacterium salmonica*, *Bacterium salmonicida*, *Bacillus truttae*, *Bacillus salmonicida and Hemophilus piscium* (Austin, 2011).

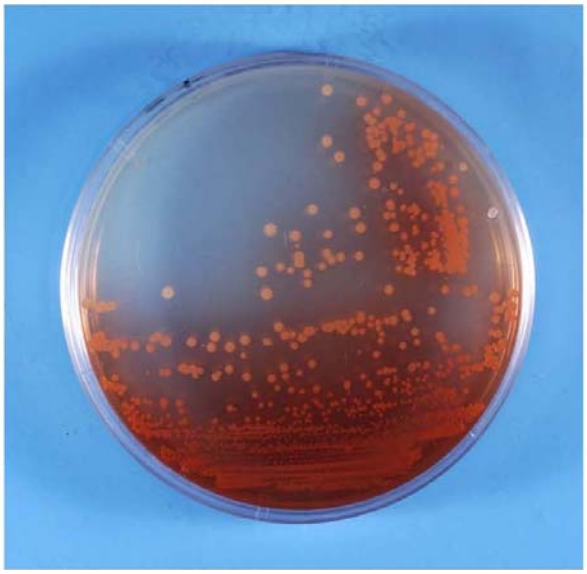

Figure 2.1. Colonies of *Aeromonas salmonicida* subsp. *salmonicida* growing on trypticase soy agar, showing water soluble brown pigment.

2.5. Genome Size

A. salmonicida subsp. *salmonicida* A449 consists of a single circular chromosome, two large plasmids and three small plasmids. The 4,702,402 bp chromosome has a G+C content of 58.5% and contains 4388 genes, with 4086 encoding proteins (Reith et al, 2008).

2.6. Serological Classification

A. salmonicida subsp. *salmonicida* can be defined as biochemically, antigenically, and genetically homogeneous with no biotypes, serotypes or genotypes being detected.

2.7. Pathogenesis

Virulence mechanisms of this pathogen fall broadly into two categories, these being cell-surface structures and extracellular products (ECPs) excreted by the cell.

Early studies of the molecular properties of *A. salmonicida* reported the presence of a special surface protein array called the A-layer or S-layer, which was responsible for the bacteria's virulent traits, and the presence of lipopolysaccharide (LPS), the cell's major cell envelope antigen. The A-layer is mainly composed of a 50Kd protein called A-protein and provides protective barrier against the defense mechanism of fish hosts. The LPS consists of three moieties; lipid A, core oligosaccharide and O-polysaccharide (o-antigen).

Since clinical signs of furunculosis are readily produced in fish injected with ECPs produced during the growth of *A. salmonicida*, an extensive body of research exists on mechanisms of virulence associated with this pathogen. The extracellular products of the pathogen consist of 25 proteins, enzymes and toxins and many more.

2.8. Diagnostic Methods

2.8.1. Clinical Signs

Furunculosis is an acute to chronic condition, with a variety of clinical signs. The disease generally appears to develop as a septicaemia and is often fatal. Affected fish often show darkening of skin, lethargy and inappetence. Haemorrhages may occur at the base of fins and the abdominal walls, heart and liver. Enlargement of the spleen and inflammation of the lower intestine are common features of chronic infections, but in acute outbreaks the fish may rapidly die without showing many signs.

Figure 2.2. Furunculosis in rainbow trout; note the furuncle cut away to show the underlying necrotic tissue.

2.8.2. Incubation Period

At 14˚C, the period from exposure of susceptible fish to this pathogen by cohabitation with infected fish to bacterial shedding can be as short as three days, death can occur as soon as two days later (i.e. at five days post-exposure). At low temperatures, the time between infection and death may be prolonged. This may be due to the effects of temperature on pathogen multiplication and host defense mechanisms (see Department of Agriculture, Fisheries and Forestry, 2009).

2.8.3. Histopathology

In sub acute/chronic infections the heart and spleen are often the most infected organs; microcolonies occur in vascular endothelia with massive destruction of spleen ellipsoids, resulting in vascular collapse; damage to spleen ellipsoids that may be accompanied by reticular cell proliferation and lymphocyte accumulation. There is degeneration of cardiac ventral epicardium and toxic cardiac necrosis, especially of the atrial lining with damage to spleen and heart.

2.8.4 Definitive Diagnosis

Presumptive diagnosis of typical *A. salmonicida* infections in salmonids is easier than the diagnosis of atypical furunculosis because clinical signs in the typical form are more stable and lesions are often not contaminated with opportunistic fungi and bacteria. Definitive diagnosis of furunculosis requires isolation and identification of the pigmented, typical strain of *A. salmonicida*. The pathogen can be readily isolated from kidney tissues of dead or moribund fishes using commercial media such as trypticase soy agar or brain-heart infusion agar plates incubated at 20-25˚C. Colonies of *A. salmonicida* subsp. *salmonicida* on these media will appear hard, friable, smooth and dark in color. After 24 hours of growth, the bacterial colonies will reach about the size of a pin point. The colonies also have a brown pigmented color that appears after they have been growing for 48-72 hours. Differentiation of colonial types that grow upon primary isolation can be facilitated by the simple addition of 0.1% (weight: volume) Coomassie Brilliant Blue (CBB) R-250 into either of the aforementioned media (CBB agar). When cultured on CBB agar, the A-layer protein that is present in virulent strains of *A. salmonicida* will absorb the CBB protein-specific dye. Consequently, virulent *A. salmonicida* develop dark blue to deep violet, friable colonies on CBB agar (Cipriano and Bullock, 2001). The API 20E rapid identification system has been widely used for identification of *A. salmonicida* subsp. *salmonicida* (Popovic et al, 2007).

2.9. Serological Identification

While cultural and biochemical characteristics produce good results, more rapid serological procedures include: serum agglutination, fluorescent antibody, or enzyme linked immune sorbent assay (ELISA) using infected tissues or isolated bacteria (Austin and Austin, 2012).

2.10. Molecular Identification

The slow growth characteristics of this bacterium, the existence of a viable, but non-culturable state, as well as the high incidence of covert infections, support the need for culture-independent, molecular diagnostic protocols (Gustafson et al, 1992). Using PCR and a specific DNA probe, the existence of *A. salmonicida* was reported in effluent, water, faces and sediment from fresh water Atlantic salmon farm. Although the highest specificity in the detection of *A. salmonicida* is obtained when the PCR assay is directed to the amplification of the surface A-layer gene, recent studies allowed the design of new primer sets targeted to the gene *fst*A (coding for an outer membrane siderophore-receptor), which showed a total specificity for *A. salmonicida* isolates (Beaz-Hidalgo and Figueras, 2012).

2.11. Control

Control methods for this disease have involved use of good husbandry practices (including good water quality, adequate disinfection of equipment and eggs, and lower stocking densities), disease-resistant fish stocks, improved diets, nonspecific immune stimulants, antimicrobial compounds, probiotics (micro-organisms that exert a beneficial effect on the host) and vaccines.

In the laboratory, typical *A. salmonicida* can be shown to be sensitive to a wide range of antimicrobial chemotherapeutants. Oral administration of these antimicrobial compounds is useful for control. Terramycin (oxytetracycline) should be added to feed at the rate of 3.0 g/100 lb fish, administered daily for 10 days to affected fish. Sulfamerazine should be administered at the rate of 5-10 g/100 lb fish and fed for 10 or 15 consecutive days (Schachte, 2002).

2.12. Prevention

Regular monitoring programs that detect *A. salmonicida* in the water supply and provide early non-lethal detection in mucus can be coupled with topical disinfection or antibiotic regimens that either preclude or minimize infection.

If eggs must be imported from outside of the hatchery system, insist that only eggs supplied from inspected and certified furunculosis-free sources be used. United States Fish and Wildlife Service regulations recommend that eggs should be disinfected by submersion for PVP-iodine after fertilization. If eggs are then shipped to another facility for incubation, policy requires that those eggs undergo a secondary disinfection using the same agent (USFWS, 1995). In practice, conduct of the stress induced furunculosis assay and regulatory confinement of infected smolts have reduced the number of furunculosis outbreaks associated with early marine culture.

2.13. Vaccine

Vaccination leads to the production of antibodies against both cellular and soluble antigens of *A. salmonicida*. Vaccination also stimulates cellular immunity. Most vaccines use oil-based adjuvants because they confer superior protection and duration of immunity compared to other adjuvants. The best results in terms of protection have been reported in salmonids with the mineral oil-adjuvanted vaccines; however, these bacterins posses several adverse side-effects. To avoid these drawbacks, new non-mineral oil-adjuvanted vaccines have been recently developed. However, recombinant DNA technology allowed the construction of highly attenuated and stable aroA auxotrophic mutant strains (using an allelic replacement technique), which were employed experimentally as safe live vaccines with high success. Approval of this method for field use has not yet been given (Press et al, 1996).

2.14. Recent topics

Although biotechnology is employed in the detection and control of furunculosis, there is still need for rapid, reliable and easy diagnostic systems suitable for covert carrier fish and field use (Austin, 1997). There is evidence to suggest that distribution and transmission of many pathogenic bacteria will increase with global warming. Few studies have tried to predict the impact of this phenomenon on *A, salmonicida* (Tam et al, 2011). Advancement in disease control measures is expected, particularly the use of probiotics, non specific immunostimulants such asβ-1, 3-Glucans and oral vaccines.

Glossary

LPS: Lipopolysaccharide
ECPs: Extracellular products
CBB: sCoomassie brilliant blue

3. BACTERIAL GILL DISEASE

Hisatsugu Wakabayashi

3.1. Synopsis

Bacterial gill disease (BGD) caused by *Flavobacterium branchiophilum* has been reported from various cultured freshwater fish species, in particular salmonids, worldwide. The bacterium is Gram-negative slender rods measuring 0.5 × 5-8 µm that are non-motile and showed neither gliding movement nor swarming growth on cytohaga agar (CA). When an outbreak of BGD occurs, *F. branchiophilum* first appears abundantly on the surfaces of the gills. A proliferative hyperplasia develops in the epithelium and progresses to clubbing and fusion of gill lamellae. Several chemical disinfectants including sodium chloride have been used to treat BGD. When the bacteria on the gills are removed by treatment at the early stage of infection, fish recover rapidly.

3.2. Introduction

Bacterial gill disease (BGD) is characterized by the presence of numerous filamentous bacteria on the surface of the gill epithelium. Davis (1926, 1927) first observed it in fry and fingerling brook trout (*Salvelinus fontinalis)* and rainbow trout (*Oncorhyncus mykiss*) in hatcheries in Vermont, USA. He called the condition bacterial gill disease, but did not attempt to isolate or identify the bacteria. Rucker et al. (1952) and Bullock (1972) isolated several strains of yellow-pigmented bacteria, referred to myxobacteria from infected gill tissue but none could be shown to be the causative agent. Kimura et al. (1978) and Wakabayashi et al. (1980) isolated a different yellow-pigmented bacterium from gill lesions of several species of salmonids in Japan and USA, and experimentally produced BGD with this organism. The bacterium was named *Flavobacterium branchiophila* by Wakabayashi et al. (1989). Later, this name was corrected to *F. branchiophilum* by von Graevenitz, (1990). BGD with *F. branchiophilum* has been reported from various cultured freshwater fish species, especially salmonids, in Japan, USA, Hungary (Farkas 1985), Canada (Ferguson et al. 1991, and Korea (Ko and Heo 1997). Probably the cause of most BGD outbreaks in salmonids is *F. branchiophilum* (Bullock 1990, Turnbull 1993). However, similar pathology could result from the presence of a low grade opportunist pathogen in extreme environmental conditions, or the presence of a highly pathogenic bacterium in marginal environmental conditions (Turnbull 1993).

3.3. Disease Agent

3.3.1. Flabobacterium Branchiophilum

Cells of *F. branchiophilum* from cytophaga broth (CB) (Anacker and Ordal 1959) are Gram-negative slender rods measuring 0.5 × 5-8 µm and usually occurred in chains of