[3] Diseases Caused By Bacterial Pathogens In Inland Water

control of channel catfish. These are florfenicol (Aquaflor), oxytetracycline (Terramycin), sulfadimethoxine-ormetoprim (Romet-30). Florfenicol is fed at 10 mg/kg of fish/day for 10 days. Oxytetracycline is fed at 50–75 mg/kg of fish/day for 12–14 days. Sulfadimethoxine-ormetoprim is also fed at 50–75 mg/kg of fish/day for 5 days. The withdrawal periods for florfenicol, oxytetracycline and sulfadimethoxine-ormetoprim are 12, 21, and 3 days prior to harvest, respectively (Evans et al, 2011).

7.5.3. Vaccine

Saeed and Plumb (1986) demonstrated enhancement of the agglutination antibody titre and protection by multiple injections of the LPS prepared from *E. ictaluri.* Similarly, it is reported that an intraperitoneal injection of a whole-cell preparation of formalin-killed *E. ictaluri,* cellular extract, and crude membrane produced agglutination antibody titres (Vinitnantharat and Plumb, 1992). Primary immunodominant antigens in the cell membrane of *E. ictaluri* with a molecular mass of 36 kDa and 60 kDa provided protection to channel catfish (Vinitnantharat et al, 1993). Thune et al. (1994) investigated the practical application of vaccination against *E. ictaluri*. They demonstrated the potential of an oral/immersion method of killed *E. ictaluri* vaccine to small size channel catfish. Recently, because of its higher efficacy, attenuated *E. ictaluri* vaccine attracts more attention than other types of vaccines. Klesius and Shoemaker (1999) developed an attenuated vaccine strain RE-33 by passages on an antibiotic of refampicin. Strain RE-33 (AQUAVAC-ESC^{TM}) has been available since 2000 for catfish farming in the USA. About 25% of all catfish fry and/or fingerlings produced in south-eastern USA have been immunized with this attenuated vaccine (Evans et al, 2011)[5].

7.6. Recent Topics

The entire genome sequence of *E. ictaluri* 93-146 has been determined (Williams et al, 2012). Yang et al. (2012) also sequenced the whole genome of *E. ictaluri* ATCC33202 to conduct comparative phylogenomic analyses of *Edwardsiella* species. They found that 93-146 and ATCC33202 share most of the genomic islands (GIs) and the insertion sequence (IS) elements between themselves, but *E. tarda* strains have higher sequence divergence of GIs and IS elements. The conserved GIs and IS element profiles in *E. ictaluri* strains imply that the genomes of different *E. ictaluri* might be kept less modified in relatively fixed hosts, whilst the variance distribution of GIs and IS elements in different *E. tarda* strains may correspond to the broad host range properties. Presence of the gene clusters of the type III and type VI secretion systems was determined in the genome of *E. ictaluri*. The importance of these secretion systems in the virulence of *Edwardsiella* species was suggested from functional studies using gene mutagenesis techniques (Thune et al, 2007; Wang et al, 2009;Chakraborty et al, 2011; Rogge and Thune, 2011).

8. MOTILE AEROMONADS DISEASE

Tomokazu Takano

8.1. Synopsis

A haemorrhagic septicaemia caused by *Aeromonas hydrophila* complex has been

observed in numerous species of fresh water fish and occasionally in marine fish and amphibians, reptiles, cattle and humans through out the world. Especially, the severe diseases occur in cultured freshwater fish (Bullock *et al*., 1971; Aoki, 1974; Egusa, 1978; Khardori and Fainstein, 1988; Schäperclaus *et al*., 1992). In this section, characteristics, diagnostic methods, and control of *A. hydrophila* are reviewed.

8.2. Introduction

Infectious abdominal dropsy in common carp has been attributed to the *A. hydrophila* group (*Aeromonas punctata*) and was first described by Schäperclaus (1930). During the 1960s, outbreaks of red fin disease, caused by *A. hydrophila*, occurred frequently in cultured eel in Japan (Hoshina, 1962; Egusa, 1978). The bacterium inhabits widely in freshwater environment such as water and bottom sediments containing organic material, as well as in the intestinal tract of fish (Aoki, 1974; Egusa, 1978; Hazen *et al*., 1978; Seidler *et al*., 1980; Kaper *et al*., 1981; van der Kooij and Hijnen, 1988; Sugita *et al*., 1994; Dumontet *et al*., 1996). Concurrent infection by *Saprolegnia parasitica* in cultured Japanese eel was also reported (Egusa, 1978). Hence, *Aeromonas hydrophila* is typically recognized as an opportunistic pathogen or secondary invader (Austin and Austin, 1987). Infection by *A. hydropila* became known in most cultured fresh water fish species. Therefore, *A. hydropila* is economically important in fresh water farming.

8.3. Disease Agent

Characteristics

Kou (1972a, 1973) and Wakabayashi *et al*. (1981) recognized that almost all pathogenic strains of motile aeromonads relevant to aquaculture were encompassed within *A. hydrophila* biover. *hydrophila*, proposed by Popoff and Véron (1976).

Aeromonas hydrophila is a Gram-negative rod-shaped bacterium and is motile, due to a monotrichous polar flagellum. The bacterium measures 0.3–3.0 μm in diameter and 1.0–3.5 μm in length. It was no spore stage or capsule. The optimum growth temperature is 28°C, but growth can occur at 27°C. Colonies on nutrient agar are white to pale pink, round and convex, with entire margins.

The biochemical characteristics are shown in Table 8.1. It is a facultative anaerobe, fermenting carbohydrates to acid, or acid and gas. *Aeromonas hydrophila* is resistant to the vibriostatic agent O/129 (phosphate: 2,4-diamino-6,7-diisopropylpteridine phosphate) 150 μg, reduces nitrates to nitrate, is unable to grow in media containing 6.5% sodium chloride (NaCl) and is generally resistant to ampicillin and carbenicillin. The guanine plus cytosine (G + C) content of the deoxyribonucleic acid (DNA) is 57–63% (MacInnes *et al*., 1979; Aoki, 1999).

Table 1. Biological characteristics of *Aeromonas hydrophila.*

Characteristics	Response
Indole production in 1% peptone water	+
Aesculin hydrolysis	+
Growth in potassium cyanide (KCN) broth	+
L-Histidine and L-arginine utilization	+
L-Arabinose utilization	+
Acetoin from glucose (Voges-Proskauer (VP) reaction)	+
H_2S from cysteine	+
Oxidase	+
Cytochrome Oxidase	+
Catalase	+
Methyl red (MR) experiment	d
Acethylmethylcarbinol production	+
2,3-Butanediol production	+
2,3-Butanediol dehydrogenase	+
β-Galactosidase production	+
Phosphatase	+
Nitrate reduction	+
Urease	−
Malonate	−
Gelatin liquefaction	+
Casein digestion	+
Loeffler serum digestion	+
Starch hydrolysis	+
Lipase	+
Lecithinase	+
Glucuronate utilization	+
Ornithine decarboxylase	−
DNAse	+
RNAse	+
Haemolysis	+
Carbohydrate decomposition	
Adonitol	−
Aesculin	d
Arabinose	d
Cellobiose	d
Dextrin	+
Dulcitol	−
Fructose	+
Galactose	+
Glucose	+
Glycerol	+
Glycogen	+
Inositol	−
Insulin	−
Lactose	d
Maltose	+
Mannitol	+
Mannose	+
Melezitose	−
Raffinose	d
Rhamnose	d
Salicin	d
Sorbitol	d
Sorbose	−
Starch	+
Sucrose	d
Trehalose	+
Xylose	−

+, Typically positive; −, typically negative; d, differs among strains;
H_2S, hydrogen sulphide; DNAse, deoxyribonuclease; RNAse, ribonuclease.

Table 8.1. Biological characteristics of *Aeromonas hydrophila.*

Classification

Aeromonas hydrophila contains thermostable O, thermolabile K and flagellar H antigens. Serologically, the O antigen of *A. hydrophila* is hetero generous (Sakazaki and Shimada, 1984; Janda *et al*., 1994, 1996). Different serotypes have been observed from various sources of fish, isolated in different years and places (Eddy, 1960; Bullock, 1966). Interestingly, a common antigen has been found among virulent strains (Kou, 1972b; Leblanc *et al*., 1981). Protein fingerprints do not correlate with biochemical characteristics. Both phenotype and protein fingerprints show clustering of epizootiologically related isolates (Millership and Want, 1993). Maruvada *et al*. (1992) detected species-specific polypeptides of the outermembrane from *A. hydrophila*, and Wilcox *et al*. (1992) suggested that outermembrane protein profiles were useful for confirming the identity of *A. hydrophila*. Shaw and Hodder (1978) showed that O-polysaccharides were remarkably similar structure in motile Aeromonas species, including *A. hydrophila*. MacInnes *et al*. (1979) investigated the DNA homology of 17 strains of *A. hydrophila*, which had been collected from various sources, using *A. hydrophila* ATCC7966 as a reference strain. The percentage homology DNA ranged from 39 to 100%, with a mean value of 64.7%. *Aeromonas hydrophila* does not seem to show any significant divergence among the 17 strains investigated. The 16S ribosomal DNA (rDNA) from ten species of *Aeromonas* was sequenced to analyze relatedness (Martinez-Murcia *et al*., 1992). Homology for 16S rDNA of the ten species exhibited very high levels, ranging 98 to 100%. East and Collins (1993) showed that region encoding 23S ribonucleic acid (RNA) from *A. hydrophila* was identical to that of gamma division of Proteobacteria, *Escherichia coli* and *Plesiomonas shigelloides*. Small-subunit ribosomal RNA (rRNA) sequences of *Aeromonas* were examined for a phylogenetic analysis (Ruimy *et al*. 1994).

Host range and Pathogenesis

Most cultured fresh water fish are susceptible to infection by *A. hydropila*, such as brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*Oncorhynchus tshawytscha*), ayu (*Plecoglossus altivelis*), carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), clariid catfish (*Clarias batrachus*), Japanese eel (*Anguilla japonica*), American eel (*Anguilla rostrata*), gizzard shad (*Dorosoma cepedianum*), goldfish (*Carassius auratus*), golden shiner (*Notemigonus crysoleucas*) and tilapia (*Tilapia nilotica*) (Bullock *et al*., 1971; Egusa, 1978: Saitanu, 1986; Aoki, 1999).

A variety of possible virulence factors of *A. hydrophila* have been suggested, including lipopolysaccharides (endotoxins), extracelluar products (ECP), siderophores, the abilitu of attachment to host cells and surface proteins. The ECP include a cytotoxin, enterotoxin, haemolysins, protease, haemagglutinin and acetyl cholinesterase (Cahill, 1990; Gosling, 1996; Howards *et al*., 1996). *Aeromonas hydrophila* enters through the epithelium of the intestinal tract of fish. Enterotoxins of *A. hydrophila* cause fluid to accumulate in ligated rabbit ileal loops. Enterotoxins are divided into two types, cytotonic and cytotoxic.

8.4. Diagnostic Methods

Isolation

Aeromonas hydrophila can be grown on brain-heart infusion medium, tryptosoy agar,

neutrient agar and MacConkey agar with incubation at 20–25°C for 24–48 h. Numerous selective media have been developed fro the isolation and presumptive identification of *A. hydrophila* or motile aeromonads (Moyer, 1996), including Rimler-Shotts medium (Shotts and Rimler, 1973), modified peptone beef-extract glycogen agar (McCoy and Seidler, 1973), Rippey-Cabelli (membrane filter method (mA)) agar (Rippey and Cabelli, 1979), MacConkey's agar supplemented with trehalose (Kaper *et al*., 1979) and starch-ampicillin agar (Palumbo *et al*., 1985). Davis and Sizemore (1981) reported that Rimler and Shotts medium and Rippey-Cabelli agar were not suitable for *A. hydrophila*. Arcos *et al*. (1988) compared six media for selective isolation of *A. hydrophila* and showed that mA agar gave the best recovery rate and also an acceptable specificity, but its selectivity was low. An API-20E test strip can be used for identification of the Enterobacteriaceae including *A. hydrophila*.

Clinical signs, Gross pathology and Histopathology

Diseased fish usually display cutaneous haemorrhage of the fins and trunk, and the condition is often referred to as 'red fin disease' (Hoshina, 1962). The bacteria multiply inside the intestine, causing a haemorrhagic mucousdesquamative catarrh. Toxic metabolites of *A. hydrophila* are absorbed from the intestine and induce a toxaemia. Capillary haemorrhage occurs in the dermis of fins and trunk and in the submucosa of the stomach. Hepatic cells and epithelia of renal tubules show degeneration. Glomeruli are destroyed and the tissue becomes haemorrhagic, with exudates of serum and fibrin (Miyazaki and Jo, 1985; Miyazaki and Kaige, 1985). European carp infected with *A. hydrophila* show severe tail and fin rot and visible haemorrhage and ulceration of the body surface. Widespread proliferation of bacteria is usually observed in the intestine. In some reports (Fijan, 1972; Wolf, 1988), the histopathological phenomena associated with the rhabdovirus infection haemorrhagic septicaemia of carp have been erroneously attributed to motile aeromonads (Bullock *et al*., 1971).

Diagnosis by serological and molecular techniques

Aeromonas hydrophila has been identified by the gel-diffusion technique (Bullock, 1966), direct fluorescent antibody technique (Lewis and Allison, 1971), indirect fluorescent antibody technique (Lewis and Savage, 1972), immunoblotted sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) (Mulla and Millership, 1993). However, these methods are of limited value, because many different serological types of *A. hydrophila* are distributed in fish farms (Eddy, 1960; Bullock, 1966). Deoxyribonucleic acid probe hybridization technology is available for the direct detection and identification of mycroorganisms. However, many common DNA fragments between *A. hydrophila* and *A. salmonicida* were reported by Miyata *et al*. (1995). Therefore, it is unlikely that this hybridization technique will be successful for this species. Cascón *et al*. (1996) found a specific PCR primer set for the detection of *A. hydrophila* hybridization group 1.

8.5. Control

Prevention

Out breaks of the disease are usually associated with a change in environmental conditions. Stressors, including overcrowding, high temperature, a sudden change of temperature, rough handling, transfer of fish, low dissolved oxygen, poor nutritional

status and fungal or parasitic infection, contribute to physiological changes and heighten susceptibility to infection. *Aeromonas hydrophila* is widely distributed in the intestinal tract of cultured fish and the water and sediments of fresh water ponds which are rich in organic materials. Virulent strains of *A. hydrophila* in these environments are possible sources of infection. Therefore, minimizing stressors, preventing overcrowding, using proper feeds, maintaining clean environment of ponds are important management practices to reduce *A. hudrophila* infection (Aoki, 1999; Cipriano and Austin, 2011).

Chemotherapy

Chemotherapeutic agents are used for the treatment of *A. hydrophila* in fish farms (Aoki, 1992). Isolates of *A. hydrophila* have been found to be sensitive to chloramphenicol, florfenicol, tetracycline, sulphonamide, nitrofuran derivatives and pyridonecarboxylic acids (Aoki and Egusa, 1971; Endo *et al*., 1973; Katae *et al*., 1979; Fukui *et al*., 1987). Fluorinated analogue, tetracycline derivatives, nitrofuran derivatives, sulphonamide and pyrodonecarboxylic acids are effective in oral treatments (Austin and Austin, 1987).

Vaccine

Experimental vaccination for prophylaxis against infection of *A. hydrophila* has been examined (Stevenson, 1988). Fish immunized either intramuscularly or intraperitoneally with vaccine showed protection against challenge. The agglutinating antibody titre increased in the serum of immunized fish (Song *et al*., 1976; Ruangpan *et al*., 1986; Karunasagar *et al*., 1991). Immersion vaccination of channel catfish using polyvalent sonicated antigens of *A. hydrophila* provides protection (Thune and Plumb, 1982). Lamers *et al*. (1985) noted that agglutinating antibody was recognized in the serum of carp immunized with *A. hydrophila* bacterin, following a second immersion with this vaccine. Catfish immunized intraperitoneally by injection with the acid extract of the S-layer protein of *A. hydrophila* were protected from the homologus, virulent strain (Ford and Thune, 1992). Serological types of *A. hydrophila* are heterogeneous and polyvalent vaccine is thought to be necessary for prevention of the infection.

8.6. Recent Topics

Draft genome sequence of *A. hydrophila* SNUFPC-A8 which was isolated from a kidney of a moribund cherry salmon (Oncorhynchus masou masou) became available. The sequence data were assembled into 59 contigs and 300 singletons. The draft genome of *A. hydrophila* SNUFPC-A8 was 4,969,090 bp in length, and a total of 4,779 open reading flames were discovered (Han *et al*., 2013). A systematic analysis of the virulence factors based on this genome database will uncover the details of the disease caused by *A. hydrophila*.

Glossary

NaCl : Sodium chloride,

DNA : Deoxyribonucleic acid,

G + C : Guanine plus cytosine,

rDNA : Ribosomal DNA,

ECP : Extracelluar products,

SDS-PAGE : Sodium dodecylsulphate-polyacrylamide gel electrophoresis

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