[3] Diseases Caused By Bacterial Pathogens In Inland Water

6. RED SPOT DISEASE

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6.1. Synopsis

Red spot disease was first reported in 1972 from farmed Japanese eel (*Anguilla japonica*) in Japan and then from farmed European eel (*A. anguilla*) in European countries, with its characteristic subepidermal petechiae on the body surface and severe mortalities. Thereafter, the disease was recorded in various non-anguillid farmed species which were reared mostly under blackish or saltwater conditions. The causative agent, *Pseudomonas anguilliseptica*, is relatively psychrophilic and halophilic, and homogeneous in the phenotypic and genetic characteristics. *P. anguilliseptica* is thought to be a typical facultative pathogen because of its low virulence in experimental infection.

6.2. Introduction

Red spot disease, or ‘sekiten-byo’ in Japanese, was first described in Japanese eel (*Anguilla japonica*) at Japanese commercial farms in 1972, and the disease had caused serious economical damages in many eel farms for successive several years in Japan (Wakabayashi and Egusa, 1972; Muroga, 1978). Typical clinical sign of the disease was subepidermal petechiae on the body surface, and the name ‘red spot disease’ was due to this conspicuous external sign (Figure 1). A histopathological examination revealed that manifestation of petechial hemorrhages in the body appeared in an advanced stage of the disease (Miyazaki and Egusa, 1977). The disease in Japanese eel was also confirmed in Taiwan in 1978 (Kuo and Kou, 1978) and then recorded in cultured European eel (*A. anguilla*) in Japan, Scotland, Denmark, France and the Netherlands (Jo et al, 1975; Stewart et al, 1983; Mellergaard and Dalsgaard, 1987; Michel et al, 1992; Haenen and Davidse, 2001). Compared with Japanese eel, European eel was relatively less susceptible to the disease (Jo et al, 1975; Haenen and Davidse, 2001).

Thereafter, the disease was recorded in various non-anguillid cultured fish species which were reared mostly under blackish or saltwater conditions in France, Spain, UK, Denmark, the Netherlands, Finland, Canada and Japan. These include black sea bream (*Acanthopagrus schlegeli*) (Nakajima et al, 1983), striped jack (*Pseudocaranx dentex*) (Kusuda et al, 1995), Atlantic cod (*Gadus morhua*) (Ferguson et al, 2004 ; Balboa et al, 2007), orange-spotted grouper (*E. coioides*) (Al-Marzouk, 1999), gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*) (Berthe et al, 1995; Domenech et al, 1997), black spot seabream (*Pagellus bogaraveo*) (Lopez-Romalde et al, 2003), salmonids such as Atlantic salmon (*Salmo salar*), sea trout (*S. trutta*), rainbow trout (*Oncorhynchus mykiss*), whitefish (*Coregonus* sp.) (Wiklund and Bylund, 1990; Wiklund and Lonnstrom, 1994) and ayu (*Plecoglossus altivelis*) (Nakai et al, 1985a). In the case of ayu, which is commonly cultured in freshwater ponds, infection might have been established in estuary where fish were caught as seeds for culture. The causative bacterium was also isolated from wild Baltic herring (*Clupea harengus membras*) with eye lesions (Lonnstrom et al, 1994).

Figure 6.1. *Pseudomonas anguilliseptica* infection (red spot disease) of Japanese eel showing intensive petechial hemorrhages on the skin

Epizootics of the disease in farmed Japanese eel in Japan prevailed mainly in early spring and sporadically in autumn when water temperature of the ponds ranged 10˚C to 20˚C, and ceased at 27˚C in early summer. Another epizootiological factor of the disease in Japanese eel farms was that farm ponds were located near the seashore and thus underground water used for fish rearing contained salinity (Cl 0.27-6.29 ppt) (Muroga et al, 1973). In cases of European eel, the disease was recorded at 23-25˚C and 16˚C in the Netherland and Denmark, respectively (Mellergaard and Dalsgaard, 1987; Haenen and Davidse, 2001). In marine fishes, the disease occurred during winter months when water temperature was below 16˚C in France and Spain (Berthe et al, 1995; Tranzo et al, 2005) or between 15˚C and 18˚C in salmonids in Finish coasts (Wiklund and Bylund, 1990). The most common clinical sign of the disease is haemorrhagic petechia on the skin. Petechial hemorrhages were also noticeable in the peritoneum and the adipose tissue of visceral organs in affected salmonids (Wiklund and Lonnstrom, 1994). The disease in Atlantic cod, Baltic herring and gilthead sea bream was often associated with eye lesions (Lonnstrom et al, 1994; Berthe et al, 1995 ; Ferguson et al, 2004).

6.3. Disease Agent

The causative agent of red spot disease, *Pseudomonas anguilliseptica*, is a Gram-negative, aerobic and motile rod, producing no acid from glucose and other carbohydrates. Growth of the bacterium on conventional agar media is rather slow and colonies are entire, convex, translucent and viscid. *P. anguilliseptica* is rather uniform in the biochemical characteristics regardless of the source of isolation, with a few exceptions. The bacterium grows in nutrient broth with NaCl 0-4% (optimum 0.5-1%) and at temperatures from 5˚C to 30˚C (optimum 15-25˚C). The cells are motile with a single polar flagellum but lose motility when cultured at 25˚C or over. Interestingly, the bacterium could survive in seawater or diluted seawater (Cl higher than 1.9 ppt) for more than 200 days, while it perished in freshwater within a day (Wakabayashi and Egusa, 1972; Muroga et al, 1977). Addition of seawater or Mg^{++} in culture media enhances motility of the cells in a wet mount method.

Electron microscopy revealed a capsule-like envelope on cell surface of *P. anguilliseptica* (Wakabayashi and Egusa, 1972). A series of serological analysis on Japanese, Taiwanese,

and Scotish isolates from eels demonstrated that the bacterium had a common heat-stable antigen (O antigen). However, based on a heat-labile antigen (stable at 100˚C for 30 min but labile at 100˚C for 120 min or 121˚C for 30 min), designated as K antigen, it was divided into two serotypes; K^+ type (K antigen-possessing) and K^- type (K antigen-lacking) (Nakai et al, 1981, 1982). These serotypes correlated well with their experimental virulence to eels; K^+ type was virulent to both Japanese and European eels but K^- type was avirulent to both species (Nakai and Muroga, 1982; Nakai et al, 1985b). *P. anguilliseptica* isolates from ayu also had K antigen (K^+-2), which was differentiated from that (K^+-1) of the eel isolate (Nakai et al, 1985a). Furthermore, it was shown that K antigen-related resistance to serum (complement)-killing of fish correlated well with the virulence of the isolates (Nakai, 1985) (Table 6.1). The Finnish isolates from salmonids were similar to serotype of the ayu isolates (Wiklund and Bylund, 1990). On the other hand, two different O serotypes were described for non-eel isolates and eel isolates; serotype O1 for isolates from turbot, sea bream, sea bass, herring and salmonids, and serotype O2 for isolates from Japanese and European eels (Lopez-Romalde et al, 2003; Balboa et al, 2007). This serotyping correlated with genotyping by randomly amplified polymorphic DNA (RAPD) analysis (Lopez-Romalde et al, 2003).

Fish or serum source	**Virulence to fish**			**Resistance to serum-killing**		
	K^+-1*[1]	K^+-2	K^-	K^+-1	K^+-2	K^-
Japanese eel	++*[2]	+	-	++	+	-
Bluegill	++	nd*[5]	-	++	nd	-
European eel	+*[3]	nd	-	+	nd	-
Ayu	+	++	nd	+	++	nd
Carp	-*[4]	-	-	-	-	-
Goldfish	-	nd	-	-	nd	-
Tilapia	-	-	-	-	-	-
Rainbow trout	-	nd	-	-	nd	-

*[1] serotype
*[2] high virulence (LD50: 10^6 cfu/fish) or high serum-resistance
*[3] low virulence (LD50: 10^{8-9} cfu/fish) or high serum-resistance
*[4] no virulence or no serum-resistance
*[5] no data

Table 6.1. Comparison of virulence to fish and resistance to serum-killing among *Pseudomonas anguilliseptica* serotypes (from (Nakai et al, 1985a; Nakai, 1985))

Several fish species were tested for their susceptibility to a virulent strain (K^+-1 type) of *P. anguilliseptica* by intramuscular injection (Muroga et al, 1975; Uno, 1976). Japanese eel was more susceptible to the pathogen than European eel. Ayu, bluegill (*Lepomis macrochirus*) and loach (*Misgurnus anguillicaudatus*) were highly susceptible, and carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) were slightly susceptible to the pathogen, while rainbow trout, amago (*Oncorhynchus rhodrus* f. *macrostoma*), kokanee salmon (*O. nerka* f. *adonis*) and iwana (*Salvelinus pluvius*) were not susceptible. A similar experimental infection with a K^+-1 type strain showed that the LD50 to Japanese eel was about 10^6 cfu/fish (Nakai et al, 1985). In a dip method, which fish were kept at

tanks containing 10^{6-7} cfu/ml of the bacterium, infection was established if diluted seawater was used as rearing water, while gastral administrations of the bacterium failed to cause mortality (Muroga and Nakajima, 1981). When Japanese eels were challenged by intramuscular injection with the K^+-1 type strain under different water temperatures, fish died at 12˚C or 20˚C with high cell numbers (10^{8-10} cfu/g or ml) in the blood and organs, but not at 28˚C (Nakai et al, 1985). The LD50 of the turbot isolate to juvenile turbot by intraperitoneal injection was 10^6 cfu/fish (Magi et al, 2009).

6.3.1. Diagnostic methods

P. anguilliseptica is easily isolated with abundant colonies on conventional nutrient agar media from various organs of diseased fish. The bacterium is biochemically homogeneous and can be differentiated from the other fish-pathogenic pseudomonads (*P. fluorescens*, *P. putida*, *P. chlororaphis* and *P. plecoglossicida*) by negative reactions in carbohydrate utilization. Serological and RAPD techniques are available for serotyping and genotyping of *P. anguilliseptica* as well as rapid diagnosis of the disease (Horiuchi and Kohga, 1979; Nakai et al, 1981; Lopez-Romalde et al, 2003). PCR-based techniques have been developed for rapid identification of *P. anguilliseptica* or sensitive detection of the pathogen from fish (Blanco et al, 2002; Romalde et al, 2004; Beaz-Hidalgo et al, 2008).

6.4. Control

P. anguilliseptica was sensitive to some antibiotics (Wakabayashi and Egusa, 1972; Wiklund and Bylund, 1990), and treatments with oxolinic acid and nalidixic acid were effective to experimentally infected Japanese eels (Jo, 1978). However, chemotherapy is not so effective in eel farms mainly due to the fact that the disease occurs in early spring when fish have poor appetite at lower water temperature. Treatment of Atlantic salmon with oxytetracycline had only a limited effect (Wiklund and Bylund, 1990).

As mentioned previously, the disease occurs preferably in Japanese eels farmed in brackish water ponds in spring and autumn. The epizootiological features were supported by the experimental results of physiological and pathological characteristics of the pathogen. Based on these findings, some control measures were proposed (Muroga, 1978). In the areas where the epizootic has been prevailing, eels should be cultured in freshwater ponds and/or the water temperature should be kept at 26˚C or higher. Particularly, the temperature manipulation was so efficacious that the epizootic had burnt low at late 1970s and completely disappeared since 1980s in eel farms in Japan. Development of green-house culture system for Japanese eel, where water temperature is constantly kept at about 26˚C for optimum growth of eel, greatly contributed to eradication of the disease.

The temperature manipulation, however, is not applicable for salmonids and other coldwater fish species or cage-cultured marine fishes in the open sea. It was confirmed under laboratory setting that both antibody response and protection in Japanese eels immunized by injection with *P. anguilliseptica* bacterin (formalin-killed cells) incorporated with FCA were maintained over five months (Nakai and Muroga, 1979). A field vaccination trial was conducted in a commercial eel farm having history of red spot

disease. Japanese eels were injected intramuscularly twice with heat-killed (100˚C for 30 min) bacterin on the beginning of November in 1980. Red spot disease occurred in the pond from May to June in 1981 and the injection vaccination procedure proved to be effective against natural infection of *P. anguilliseotica* (Nakai et al, 1982). However, any successful results have not been obtained by either immersion or oral vaccination for eels. On the other hand, it was shown that non-mineral oil-adjuvanted bacterins were effective against experimentally induced disease in gilthead seabream and turbot (Tranzo et al, 2005).

The aforementioned epizootiological and pathological findings suggest that *P. anguilliseptica* is ubiquitous in salt or brakish waters, and wild fishes might serve as an important infection source, either vector or carrier, for farmed fish (Lonnstrom et al, 1994). Since *P. anguilliseptica* is possibly a typical facultative pathogen to any fish species, it is essential to reduce predisposing factors for controlling the disease (Mushiake et al, 1984).

7. EDWARDSIELLOSIS (EDWARDSIELLA ICTALURI)

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7.1. Synopsis

Edwardsiella is a distinct taxon within the family Enterobacteriaceae, and includes three species, *Edwardsiella ictaluri* (Hawke, et al, 1981), *E. tarda* (Ewing et al, 1965) and *E. hosinae* (Grimont et al, 1980). *Edwardsiella hosinae* strains were mainly isolated from birds and reptiles (Grimont et al, 1980). Both *E. ictaluri* and *E. tarda* cause diseases in fish. More specifically, *E. ictaluri* is associated with freshwater fish species including ictalurid fish, whilst *E. tarda* has a broader host range amongst freshwater and marine fish species (Abbott and Janda, 2006; Evans et al, 2011). In this section information on *E. ictaluri* is discussed.

7.2. Introduction

Hawke (Hawke 1979) first reported undefined species of *Edwardsiella* from channel catfish (*Ictalurus punctatus*) suffering from enteric septicaemia. His later research (Hawke, et al, 1981) revealed that *Edwardsiella ictaluri* was the causative agent of enteric septicaemia of catfish (ESC), which is one of the most important infectious diseases of the catfish industry in the USA. The economic losses caused by ESC have been estimated to be US$20-60 million/year (Evans et al, 2011; Plumb and Vinitnantharat, 1993; Shoemaker et al, 2003). Besides catfish production in USA, the freshwater catfish (*Pangasius hypophthalmus*) industry in Southeast Asian countries also suffers from *E. ictaluri* infections (Ferguson et al, 2001; Crumlish et al, 2002 ; Yuasa et al, 2003).

7.3. Disease Agent

7.3.1 Characteristics

The type strain of *E. ictaluri* is ATCC 33202. It is a Gram-negative, rod-shaped bacterium which measures 0.5 by 1.25 μm after 18 to 48 h of culture on solid media. At 25°C it is