[4] Diseases Caused By Bacterial Pathogens in Saltwater

infection was effective in cultured turbot. High degrees of protection lasted for at least a year (Toranze *et al*., 1995). In vaccine trials in flounder, formalin-killed cells of both serotypes (I and II) were effective against challenges with a homologous serotype strain, whereas the vaccine efficacy against the heterologous serotype was not consistent in the mortality and reservoir rates (Mori *et al*., 2012). A combined formalin-killed serotype I and II vaccine is licensed for use in flounder in Japan.

1.4.5.3 Probiotics

Probiotics including *Lactobacillus plantarum*, *L. acidophilus*, *L. brevis*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* and herbal mixture supplementation diet enhance growth, blood composition, and nonspecific immune response to experimental infection with *S. parauberis* in flounder (Harikrishnan *et al*., 2011).

The *L. sakei* BK19 supplemented diet (10^8 cells g^{-1}) fed to grouper, *Epinephelus bruneus* for two weeks reduced the mortality in the experimental challenge with *S. parauberis* as compared to the non-supplementated group. The immune response in probiotics fed group showed significantly increased phagocytic and peroxidase activities (Harikrishnan *et al*., 2010).

1.4.6. Recent Topics

S. parauberis was recovered from a spoiled vacuum-packaged refrigerated seafood product. Isolates were identified by 16S rRNA gene sequencing and characterized using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The MALDI-TOF MS allowed rapid and direct identification of *S. parauberis* (Fernandez-No *et al*., 2012).

Glossary

MALDI-TOF MS: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

2. NOCARDIOSIS

Masahiro Sakai

2.1. Synopsis

Nocardiosis caused by the bacterium, *Nocardia seriolae*, has made serious damage in Japanese mariculture. Typical disease signs appear as nodules in gills, spleen, kidney and liver. Although the progression of the disease is chronic and slow, the mortality rate may reach 50% or more. As this bacterium is believed to be intracellular, it is difficult to effectively treat with drug administration. Therefore, the development of effective vaccines against nocardiosis is necessary.

2.2. Introduction

Nocardiosis in fish was first described by Rucker (1949) as *Streptomyces salmonicida*

infection in sockeye salmon (*Oncorhynchus nerka*). This bacterium was identified as *Nocardia salmonicida*, based on the presence of meso-diaminopimelic acid, arabinose and galactose in the whole organism hydrolysates. The outbreak of nocardiosis was first reported from cultured marine fishes such as yellowtail (*Seriola quinqueradiata*) in Mie Prefecture, Japan, in 1967 (Kariya et al, 1968) and then it spread to fish farms in the western districts of Japan. This disease is characterized by the formation of abscesses in the epidermis and of tubercles in gills, kidneys, and spleens (Kariya et al, 1968; Kusuda and Taki, 1973). In 1968, the causative organism was isolated and proposed as a new species, "*Nocardia kampachi*". After that, Kudo et al. (1988), on the basis of deoxyribonucleic acid (DNA)-DNA hybridization and mycolic and fatty acid profiles in addition to physiological and biochemical characteristics, proposed a new species, *Nocardia seriolae*.

At present, this infection is feared as the greatest damage causing disease in yellowtail and amberjack aquaculture. Outbreaks of nocardiosis in the farms usually initiate in September or October and terminate in November. These results suggest that infection of *N. seriolae* in yellowtail begins between July and August (Itano et al, 2008).

2.3. Characteristics of the Disease

The internal pathology of nocardiosis is easily confused with other white-spot-forming diseases, such as mycobacteriosis (fish tuberculosis) and photobacteriosis (formerly Pasteurella or pseudo-tuberculosis), especially if mixed infections exist. The white-yellow granulomas are usually 1-2 mm in size. The spots are most obvious in the spleen, kidney and liver but can be found in any tissue. Fish mount a significant immune reaction and exhibit hard black spots (melano-macrophage accumulations) in place of the white spots in the liver and adipose tissues. Brown-black crusty plaques often develop on the dorsal inner surface of the swim bladder (Sheppard, 2005). Typical disease signs include nodules in gills, spleen, kidney and liver with or without multiple skin ulcers/abscesses (Austin and Austin, 2007). Histo-pathologically, the observed lesions are typical granulomas (Egusa, 1983). Cornwell et al. (2011), reported that fish infected with *N. seriolae* had necrotic external lesions on the caudal peduncles, the lower jaw, the flank near the base of the left pectoral fin, and the dorsal skull. Internally, infected fish has multiple raised white foci on the posterior kidney.

2.4. Disease Agent

The causative agent of Nocardiosis is *Nocardia seriolae*. This bacterium is a Gram-positive, acid-fast, aerobic, non-motile, pleomorphic rod-shaped bacterium (Figure 2.1). The morphology of *N. seriolae* varies, but cells are generally filamentous, branched or beaded. The bacterium is acid-fast and can grow on a variety of media containing carbon and nitrogen sources. The isolates of *N. seriolae* contain meso-diaminopimelic acid, arabinose and galactose, suggesting chemotype IVA. Iso- and anteiso-branched acids have not been detected. The total number of carbon atoms in the mycolic acids is from 44 to 58. The predominant isoprenoid quinone is tetrahydrogenated menaquinone with eight isoprene units. The G + C ratio of the DNA is 66.8-67.4 mol % (Austin and Austin, 2007).

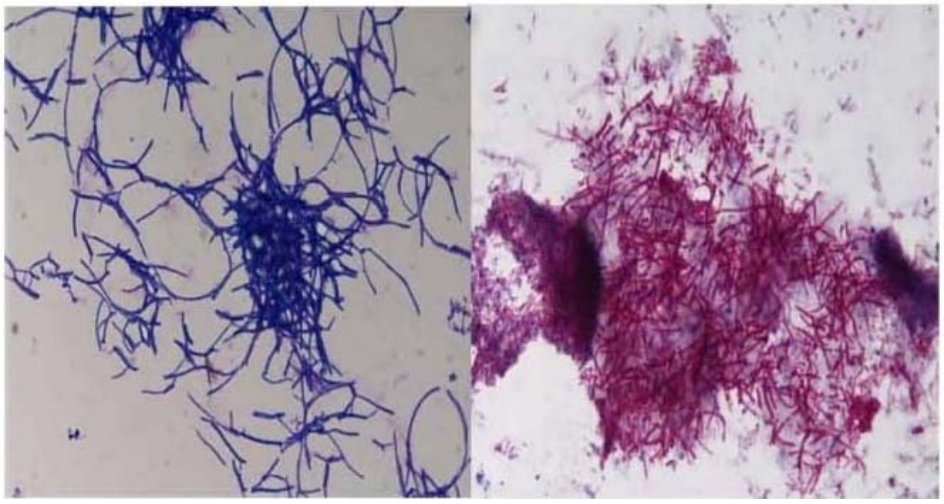

Figure 2.1. Gram-stain from infected fish showing typical branching Gram-positive hyphae indicative of nocardial infections (X1000). **b:** Ziehl-Neelsen stain from infected fish showing typical acid-fast branching hyphae (X1000). (Photos courtesy of Prof. T. Yoshida and Dr. TFIM Ismail)

2.5. Diagnosis

Figure 2.2 shows the typical fish nocardiosis signs with multiple focal or diffuse skin ulcers (upper) and spleen (lower). The typical external symptoms are: thin fish, skin nodules (focal, multifocal or coalescing), skin ulceration, opercular erosion and irregularly-shaped fleshy white masses at the base of the gill filaments (Sheppard, 2005). The internal pathology of nocardiosis is easily confused with other white-spot-forming diseases, such as mycobacteriosis and photobacteriosis. The white-yellow granulomata are usually 1-2 mm in size. The spots are most obvious in the spleen, kidney and liver but can be found in any tissue.

N. seriolae as causative agent can be isolated on brain heart infusion agar (BHIA), tryptone soya agar (TSA) and nutrient agar (NA), with optimum growth at 20–30°C temperature (Kusuda and Taki, 1973). It produces flat, wrinkled colonies after 10 days at 25°C.

The molecular method using polymerase chain reaction (PCR) and Loop-mediated isothermal amplification (LAMP) has been developed and used for detection of *N. seriolae* infection in fish (Kono et al, 2002; Miyoshi and Suzuki, 2003; Itano et al, 2006). Itano et al. (Itano et al, 2006) reported that the detection of *N. seriola* using LAMP was found to be more sensitive than that by PCR.

Figure 2.2. Typical disease signs of nocardiosis include multiple focal or diffuse skin ulcers (upper), and spleen (lower left). (Photos courtesy of Prof. T. Yoshida and Dr. TFIM Ismail)

2.6. Control

The use of chemotherapeutics for the treatment of nocardiosis has been reported (Yasumoto and Yasunaga, 1986; Hatai et al, 1984). Yasumoto and Yasunaga (Yasunaga, 1986) also reported that the administration of erythromycin (EM) or spiramycin (SPM) was not effective in treating yellowtails that were naturally infected with nocardiosis. However, Hatai et al. (Hatai et al, 1984) reported that EM was effective for the treatment of yellowtails that were artificially infected with *N. kampachi*, although SPM, chloramphenicol (CP), oxolinic acid (OX), and sulfamonomethoxine (SMM) were not. Itano and Kawakami (Itano and Kawakami, 2002) reported that several *N. seriolae* strains show resistant to EM and SPM. Due to emergence of these drug-resistant bacteria it is very difficult to treat the disease.

Vaccination may be the most effective method to prevent the disease. However, to date, no effective vaccine against this infection has been developed. Shimahara *et al.* (Shimahara et al, 2005) reported that no protective effects resulted from immunization with formalin-killed *N. seriolae* cells or formalin-killed cells with Freund's incomplete adjuvant, even though antibody levels increased. Itano et al. (2006) attempted to use phylogenetic relatedness and antigenic cross-reactivity to identify vaccine candidates against *N. seriolae*. The authors used environmental *Nocardia* species and evaluated the ability of these isolates (*N. soli* and *N. fluminea*) to induce protective immunity against *N.*

seriolae in injected yellowtail. Unfortunately, the isolates provided minimal protection to a challenge with virulent *N. seriolae*.

2.7. Conclusion

In Japanese marculture, nocardiosis is a disease most difficult to control. No effective drug or a vaccine has so far been developed. Therefore, the only prophylaxis is to remove the infected fish, and not to give stress to fish. In the future, development of effective vaccine against this disease is much anticipated.

Glossary

BHIA: Brain heart infusion agar,

PCR: Polymerase chain reaction,

LAMP: Loop-mediated isothermal amplification,

3. MYCOBACTERIAL DISEASE

Kim D. Thompson and Alexandra Adams

3.1. Synopsis

Fish mycobacteriosis (or fish tuberculosis), caused by *Mycobacterium* spp., is a progressive disease affecting a wide range of wild and cultured marine and freshwater fish species. The economical losses experienced by the aquaculture industry due to mycobacteriosis, the lack of effective treatment regimes and the zoonotic nature of the bacteria involved, highlight the need for rapid methods to detect and identify the bacterial species associated with disease.

Detection and identification of the mycobacteria is traditionally based on histopathology, culture, and biochemical properties, although these do not offer the sensitivity or specificity of nucleic acid-based amplification methods. As with other bacterial pathogens, DNA-based techniques have revolutionised the identification and classification of the mycobacteria. *M. marinum*, *M. fortuitum* and *M. chelonae* are the species most predominantly associated with mycobacteriosis. However, the recent use of genotyping-based techniques has resulted in an increase in the identification of a number of new *Mycobacterium* spp. associated with mycobacteriosis, and highlighted problems with polymerase chain reaction (PCR) amplification due to cross-reactions of species-specific primers with closely related mycobacteria spp.

3.2. Introduction

Mycobacteriosis, caused by non-tuberculosis mycobacteria (or atypical mycobacteria), has been reported in a wide range of freshwater and marine fish species, and can result in significant economic losses to the aquaculture industry. Three species of *Mycobacterium*, *M. marinum*, *M. fortuitum* and *M. chelonae*, have been cited as the main species involved in these infections, although various other non-tuberculous mycobacteria, including a