[4] Diseases Caused By Bacterial Pathogens in Saltwater

formation, nonfimbrial adhesions, invasions and hemagglutinins, chondroitinases, hemolysins and iron scavenging systems were also identified from the genome. More recently, Yang et al. (2012) conducted comparative phylogenomic analyses of Edwardsiella species. From the genome-based phylogenetic analysis, they described two kinds of genotypes of EdwGI and EdwGII amongst six different E. tarda strains. The Edwardsiella tarda strain, which was virulent in fish, was classified into EdwGI. Surprisingly, E. tarda EdwGI strains were clustered together with the E. ictaluri lineage, and both bacterial lineages possessed highly conserved T3SS and T6SS genes, whilst avirulent E. tarda EdwGII strains lost most of the T3SS and T6SS orthologs. T3SS and T6SS of Edwardsiella species play a crucial role in the host-pathogen interaction (Tan et al, 2005; Okuda et al, 2009; Zheng and Leung, 2007; Wang et al, 2009; Rogge and Thune, 2011). Hence, it is likely that T3SS and T6SS of E. tarda EdwGI and E. ictaluri were evolutionally essential factors to adapt to their hosts. Genome-based information of Edwardsiella species will uncover the mechanisms of their pathogenicity and may facilitate the development of prophylactic and therapeutic methods.

Glossary

ATCC : American Type Culture Collection, PCR: Polymerase Chain Reaction

8. BACTERIAL HEMOLYTIC JAUNDICE

Takaji Iida

8.1. Synopsis

Bacterial hemolytic jaundice is a disease affecting cultured yellowtail, *Seriola quinqueradiata*, in Japan. Because of high total bilirubin concentrations through hemolysis by the causative bacterium, the diseased fish exhibit yellow coloration of the skin and muscle. The bacterium is a new genus and species belonging to the family Flavobacteriaceae. PCR using primers specific for this bacterium was developed for diagnosis of this disease. Chemotherapy was shown to be efficient in controlling the disease and recent results suggest that an effective vaccine is expected to be produced.

8.2. Introduction

Since 1980, a disease called “jaundice” has been known to affect cultured yellowtail, *Seriola quinqueradiata*, in Japan. This disease is prevalent during summer to autumn among mainly two year-class fish with mortality reaching up to 20%. The diseased fish exhibit yellow coloration of the skin and muscle, low hematocrit values and high total bilirubin concentrations. Initially, some factors such as environmental or nutritional stresses were proposed as the cause of this disease. In blood smears from diseased fish stained with Giemsa, thin rod-shaped organisms were found. Injection of the blood from the diseased fish to healthy individuals induced jaundice, indicating the infectious nature of the disease. An organism was isolated from the diseased fish, and injection or bath immersion using the isolate developed the jaundice in yellowtail. The isolate possessed a single cell with a cell wall and an inner membrane, without a nuclear membrane, indicating that it is a bacterium. These results confirmed that this disease was caused by bacterial infection (Sorimachi *et al.*, 1993), and later referred to as “bacterial hemolytic

jaundice" (Maeno *et al.*, 1995). To date, this disease has been reported only in cultured *S. quinqueradiata*, in Japan.

8.3. Causative Agent

The bacterium is a Gram-negative, cytochrome oxidase-positive and catalase-positive rod, 4~6 µm in length and 0.3 µm in width. The live cells of the bacterium exhibit crawling motility, but possess no flagella and pili (Sorimachi *et al.*, 1993). Culture media without FBS cannot support the growth of the bacterium. The bacterium grow in L-15 medium and Eagle MEM with FBS, with the former yielding better growth. The bacterium grow at temperatures of 20-26ºC (optimal: 23-26ºC), NaCl concentration of 0.8-3.2% (optimal: 1.6-2.0%) and pH of 6.0-8.5 (optimal: 7.0-7.5). The bacterium can be inactivated in distilled water within 3 h, and in sea water within 5 d. Survival in 1/3 sea water is longer than in 0.85% NaCl solution, and there is no difference between survival of the bacterium in 1/3, 1/2 and 1/1 sea water. The bacterium exhibited hemolytic activity against not only yellowtail red blood cells but also horse red blood cells. However, any extracellular hemolytic factors were not detected. The mechanism of the hemolysis has not been fully investigated. Although detailed studies of serotypes have not been performed, the bacterium has at least a common antigen.

8.4. Diagnostic Methods

Diseased fish display symptoms such as yellow coloration of the skin and discoloration of the gills. Yellow coloration of the muscle and enlarged spleen are commonly observed under autopsy (Figure 1). There are low hematocrit values, and high plasma hemoglobin and total bilirubin concentrations in the blood. Histopathologically, severe anemia and necrosis in the splenic hematopoietic tissues, and degeneration and necrosis in the renal tubules and hematopoietic tissues are observed. Thin rod-shaped bacteria are frequently detected in the spleen and kidney (Maeno *et al.*, 1995). The bacteria are also easily observed in blood smears stained with Giemsa (Figure 2). A set of specific PCR primers for the 16S rDNA gene of the bacterium was reported for diagnosis of this disease (Mitsui *et al.*, 2004).

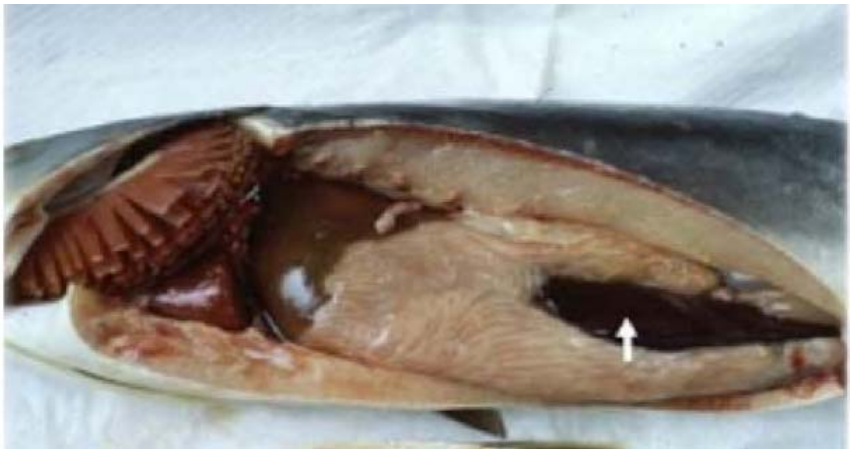

Figure 8.1. Yellowtail infected with bacterial hemolytic jaundice showing discoloration of gills and enlarged spleen (arrow).

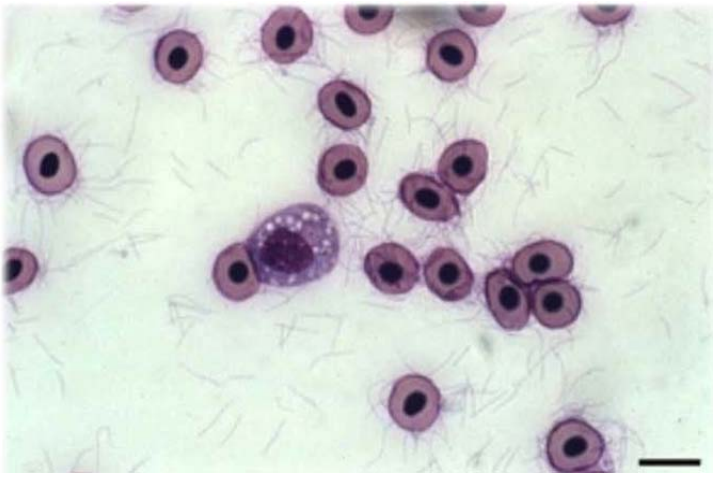

Figure 8.2. Bacterial cells in a blood smear with Giemsa stain. Scale bar: 10 um

8.5. Control

The bacterium was highly sensitive to drugs such as oxytetracyclin, tetracycline, ampicillin and erythromycin. In fish intraperitoneally injected with the bacteria and treated with various antimicrobials, more than 80% of the drug-administered group survived, while the control group only has 10% survival rate (Figure 3) (Sorimachi and Maeno, 1993). This suggests that chemotherapy is effective in controlling the disease. Fish that survived the initial artificial challenge also survived the subsequent infection challenge, and produced antibody against the bacterium (unpublished data). From these results, an effective vaccine is expected to be produced. Since mass culture of the bacterium is very difficult, genetically engineered vaccine is now being studied.

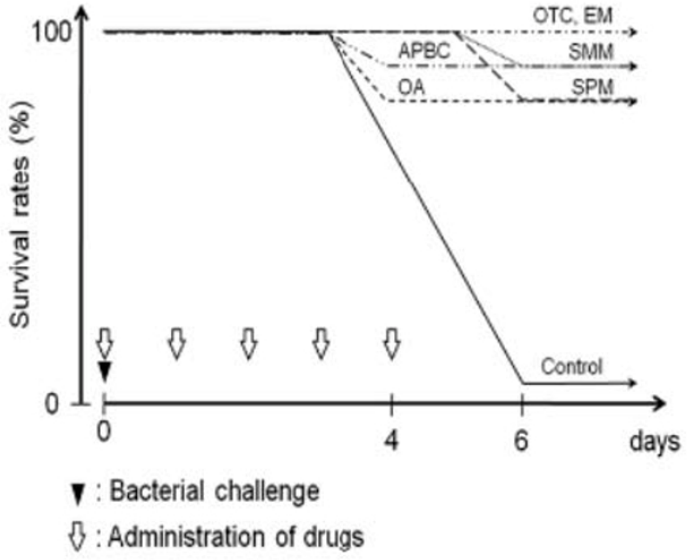

Figure 8.3. Effects of oral administration of drugs in yellowtail fry challenged with bacterial hemolytic jaundice. Abbreviation: ABPC, ampicillin; EM, erythromycin; OA, oxolinic acid; OTC, oxytetracycline; SMM, sulfamonomethoxine; SPM, streptomycin. (This figure is cited from Sorimachi and Maeno, 1993)

8.6. Recent Topics

Analysis of the 16S rDNA sequence shows that the bacterium is a member of the family Flavobacteriaceae, but does not belong to any existing genus in the family. *Icthyobacterium seriolicida* gen. nov., sp. nov. has been proposed as the name of the bacterium (in preparation).

Glossary

rDNA : Ribosomal DNA,

ABPC : Rmpicillin,

EM : Erythromycin,

OA : Oxolinic acid:

OTC : Oxytetracycline,

SMM : Sulfamonomethoxine,

SPM : Streptomycin

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