Studies on the Pathogenicity and Serological Properties of the Fish Pathogen *Tenacibaculum maritimum*

Graduate School of Fisheries Science and Environmental Studies

Tanvir Rahman

Tenacibaculum maritimum is a Gram-negative, gliding marine bacterium that causes tenacibaculosis, an ulcerative disease of marine fish around the world. The pathology of the disease has mainly been associated with characteristic gross lesions on the body surface of fish such as ulcers, necroses, eroded mouth, frayed fins and tail rot, and sometimes necroses on the gills and eyes. Despite the significance of *T. maritimum* in Japanese aquaculture, especially on Japanese flounder *Paralichthys olivaceus*, relatively little is known about its pathogenicity, and no vaccine is still available to prevent the disease. The present study is planned to carry out a detailed study on the serological characterization of *T. maritimum* and pathogenicity assessment on Japanese flounder.

First of all, a non-gliding strain of *T. maritimum* was characterized. Although the bacterium usually forms rhizoid colonies on agar media, we isolated *T. maritimum* that formed slightly yellowish round compact colonies together with the usual rhizoid colonies on an agar plate from a puffer fish *Takifugu rubripes* suffering from tenacibaculosis, and studied the biological and serological characteristics of a representative isolate of the compact colony phenotype, designated strain NUF1129. It revealed that the strain was non-gliding and avirulent in Japanese flounder in immersion challenge test, reduced in adhesion to glass wall in shaking broth culture and to the body surface of flounder and lacked a cell-surface antigen, designated antigen X, common among gliding strains in gel immunodiffusion tests using sonicated cell extracts as antigens. SDS-PAGE analysis of sonicated cell extracts showed different polypeptide banding patterns between NUF1129 and gliding strains. Like gliding strains, NUF1129 exhibited both chondroitinase and gelatinase activities, which are potential virulence factors of the bacterium. These results suggest that some cell-surface components related to gliding and adhesion ability are implicated in the virulence of *T. maritimum*.

The second was an experiment conducted to isolate antigen X from the pathogenic strain, *T. maritimum* NUF1128. The partial purification of antigen X was succeeded by hydrophobic interaction chromatography with. Polyacrylamide gel electrophoresis (PAGE) analysis of partially purified antigen X showed distinctive expression of a larger molecular weight single protein band an it was detected as immunogenic by western blot.

SDS-PAGE study revealed that antigen X is a high molecular weight protein consists of two polypeptide chains.

In the third experiment, a pathogenicity test was conducted using Japanese flounder after abraded the skin with cotton swabs or steel blades or cut off the tips of dorsal fin with scissors. Pretreated fish were immersed for 30 min in seawater containing 10^6 CFU/mL of cultured NUF1128 or NUF1129 cells and reared for 7 days. To investigate the kinetics of infection, two groups of fish abraded with cotton swabs or steel blades were challenged as above, and the viable counts of *T. maritimum* from the abraded skin were assessed at 30 min, 2 h, 6 h, 24 h and 48 h post infection. The skin tissues were examined for the bacterial proliferation by immunohistochemistry. The pathogenicity test resulted in 100% mortality of fish pretreated with steel blades and scissors and challenged with NUF1128. NUF1129 was unable to induce infection regardless of treatments applied. Infection kinetics and immunohistochemical studies revealed that NUF1128 adhered more readily than NUF1129 at exposed dermal connective tissues and proliferated exponentially associated with mortalities.

The fourth experiment was undertaken to determine the antibody titers of a rabbit anti-*T. maritimum* NUF1081 serum against formalin-killed (FKC) and heat-killed cells (HKC) of the *T. maritimum* strains with a view to find out serological variations among Japanese *T. maritimum* strains isolated from diseased Japanese flounder and puffer fish. Using unabsorbed serum, microtitre agglutination tests showed that titers of the antiserum against the strains from diseased flounder were 8192-65536 for FKCs and 32-1,024 for HKCs. Absorbed serum showed loss of agglutinability for HKC of several strains along with existence of moderate to high titer for other strains. Four strains from diseased puffer fish showed a titer range of 8,192-32,768 for FKCs and 128-512 for HKCs with unabsorbed antiserum and seemed to be serologically homogeneous except NUF1129 which showed the lowest titers, *i.e.,* 8 for FKC and 16 for HKC. Thus, different serotypes may exist among the *T. maritimum* strains isolated from diseased Japanese flounder and puffer fish.

Overall, the cell-surface components related to gliding and adhesion ability are implicated in the virulence of *T. maritimum.* The isolated partially purified surface antigen is an immunogenic high molecular weight protein which consists of two polypeptide chains. The gliding strain was found highly pathogenic for Japanese flounder, adhered more readily at dermal connective tissues through the abraded points and proliferated exponentially associated with mortalities. Different serological groups exist among Japanese *T. maritimum* strains which should be considered in future to develop vaccines.