Molecular and Antigenicity Characterisation of *Vibrio sp*. Isolates from Asian Seabass *(Lates Calcarifer)*

Siti Zubaidah Zanal Abiddin, [1]Md Sabri Mohd Yusoff & [1]Shahirudin Shamsudin

[1]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine, Universiti Putra Malaysia

Abstract

Two species of *vibrio* (*Vibrio fluvialis* and *Vibrio mimicus*) isolated earlier from *Lates calcarifer* was sub-cultured into thiosulfate citrate bile sucrose (TCBS) agar. They were then grown into brain heart infusion broth (BHI) with the addition of 1% sodium chloride (NaCl). This culture was incubated with gentle shaking (50 rpm) for 24 h at 37ºC. Outer membrane protein (OMP) of both vibrio species was prepared from the culture in brain heart infusion broth. The bacteria were pelleted and subjected to sonication to break the bacteria cells from its membrane. The outer membrane of the vibrio species was then separated using sodium polyacrylamide gel electrophoresis (SDS-PAGE) in running buffer and subjected to 300 ma, 100 v for 1 h and 20 min. The gel containing polypeptides was then transferred into a nitrocellulose membrane for immunoblotting. Hyperimmune serum against the OMP of the vibrio *spp*. raised rabbits was used in this study. Immunodetection was done by subjecting the nitrocellulose membrane to the antiserum against the respective vibrio species. Horseradish peroxidase (HRP) was used as conjugate to facilitate binding of antigen antibody complex reaction at the nitrocellulose membrane. All photographed gels were scanned and analysed using gel analysis software gene tool. The results were compared to protein standard markers. This study shows that for *vibrio fluvialis*, the most antigenic OMPs were of molecular weights 50, 60a and 75 kda whereas the most antigenic proteins for the whole cells of this strain were of 33 and 75 kda. For *vibrio mimicus* the most antigenic OMPs were of molecular weight 40 and 80 kda while the most antigenic protein for the whole cells were of molecular weights 24 and 35 kda. These antigenic proteins can be good vaccine candidates against vibrio *spp* infections.

Keywords: outer membrane protein, vibrio spp, Asian seabass, sodium polyacrylamide gel electrophoresis (SDS-PAGE), nitrocellulose membrane, immunodetection