DEVELOPMENT OF *VIBRIO ALGINOLYTICUS* VACCINE: PATHOGENESIS, IMMUNOLOGICAL AND MOLECULAR CHARACTERISATIONS

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Introduction

Vibriosis is considered to be a saltwater fish disease but freshwater fish are affected occasionally (Plumb, 1994). The common causative agent of this disease in the tropics is *Vibrio alginolyticus*, while other species affected fish in the temperate. Vibriosis in siakap, *Lates calcarifer*, has been a serious problem to cage culture fish farmers in Malaysia. The treatment of clinical vibriosis is available only for temperate species. The available vaccine for the temperate species varied in effectiveness. The objectives of the work include screening for the most virulent isolate of *V. alginolyticus* from diseased siakap. The virulent isolate was determined by pathogenicity study and pathological changes are observed. The antigens of the most virulent strain are screened for immunogenicity. The most immunogenic antigen is determined for it's ability to afford protection. Various route of immunogen administration were tested. Clonal relationships within isolates are termined molecularly. The final purpose of the research is to develop a protective vaccine against *V. alginolyticus.*

Materials and Methods

Pathogenicity study: Five strains of *V. alginolyticus* isolated from diseased siakap were determined for virulence in healthy fish. Each strain corresponding to concentrations of 0.2, 0.4, 0.6 and 0.8 optical density at Abs $_{550\ nm}$ were prepared. Descaled healthy fish were immersed in each suspension. Mortality was determined over 10 days. Pathological changes were determined by Transmission and Scanning Electron Mocroscopy. Immunogenicity study: Antigens (formalin killed cells and lipopolysaccharide) from the most virulent, moderately virulent and weakly virulent isolates were exposed to healthy fish by immersion and injection. Antibody titers of fish against the various antigens were determined. Effectiveness of immunogen was determined by challenging immunized fish with virulent *V. alginolyticus*. Molecular study: Lipopolysaccharide (LPS) was subjected to SDS-PAGE for elucidating banding patterns. DNA from 15 *V. alginolyticus* isolates were extracted and genomic finger printing was obtained by the Arbitrarily Primed Polymerase Chain Reaction (AP-PCR). Genetic relatedness between isolates was analysed by RAPDistance software downloaded from internet.

Results and Discussion

The pathogenicity study showed that all isolates tested caused fish mortality at different rates. Two strains caused 100% mortality at concentration as low as 0.2 OD. These are the two most virulent isolates. The moderately virulent isolate caused 90% mortality at 0.2 OD. The weakly virulent isolates caused 80% mortality. Gross lesion of experimental fish included hemorrhages of skin. Bacteriological sampling of organs of recently dead fish showed bright yellow colonies on TCBS, confirming *V. alginolyticus* infection. TEM of the gills of fish exposed to the bacteria showed degenerated chloride cells indicating necrosis. The ruffled edges, distorted lamellae and cylindrical shaped cells depicting bacterial cells were seen by SEM. In the immunogenicity study using formalin-killed cells and lipopolysaccharide as antigens, different rates of antibody titers were observed. Antibody titers of fish receiving formalin-killed cells of two isolates that were considered virulent and moderately virulent achieved titers of at least 1024. Fish immunized with LPS had mean antibody titers of 128. Based on these antibody titer values, both antigens can be considered to be immunogenic. When immunized fish were challenged with virulent strain, slightly lower rate of mortality when compared to unimmunized fish was seen, indicating protection. The immunogenicity of LPS layer was also correlated to the structural morphology of the substance as illustrated in the SDS-PAGE profile. The LPS of isolates that produced different pathogenesis showed different ladder-like banding patterns. All three isolates showed heterogeneous chain length of O-polysaccharide. However, among the three isolates, the most virulent isolate showed the presence of O-polysaccharide bands in the higher molecular weight region than the moderately virulent isolate. The bands of the weakly virulent isolate were in the low molecular weight region. According to a study by Velji et al. (1992), the size of the O-polysaccharide components of the LPS is an important factor determining immunogenicity. The LPS of the highest molecular weight yielded highest protection, indicating more immunogenic than LPS having low molecular weight components. Dooley et al. (1985) stated that a strong immunogenic LPS has a homogeneous O-polysaccharide chain length of the high molecular weight component. Accordingly, the most virulent isolate in this study had heterogeneous O-polysaccharide chain with some high molecular weight components, depicting a moderately immunogenic isolate. Molecular analysis based on AP-PCR showed polymorphisms in the DNA make-up of all isolates, and clonal relationship within isolates varied in genetic distance.

Conclusions

All isolates of V. alginolyticus studied were virulent but of different rates. Antigens from the most virulent isolate, namely, the formalin-killed cells and the lipopolysaccharide layers are immunogenic. Fish immunized with these antigens do afford protection as seen in the challenge study; mortality was lower in immunized than unimmunized fish. The structural morphology of the LPS layer influenced the immunogenic property of isolates. Therefore, isolates having LPS of high molecular weight, can be a good antigen for vaccine production with modification.

References

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