Invasion of green fluorescent protein-tagged *Flavobacterium columnare* in embryos of zebrafish (*Danio rerio*) and identification of protective antigens of *F. columnare*

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Abstract

Flavobacterium columnare is the pathogen of columnaris disease which causes heavy economical losses for the aquaculture industry. In order to characterize the invading procedure of the pathogen in embryos of zebrafish (*Danio rerio*) and identify the candidate protective antigen, some researches were conducted.

To characterize the invading procedure, green fluorescent protein (GFP)-tagged *F. columnare* was used to reveal the portal of entry in zebrafish embryos. Plasmid pAS43 [1] containing the *gfp* gene was transformed into *F. columnare* G_4 by conjugation to generate strain G_4g. The zebrafish embryos were infected via immersion with G_4g at 2×10^6 CFU/mL for 5 min. The infected embryos were sampled and fixed in 4% paraformaldehyde (PFA) every one hour and visualized using confocal microscopy. The result indicated that G_4g attached to the membrane of zebrafish embryos at first hour after infection and to the head of the embryos at 16 hours post infection (hpi). These infections could spread throughout the body 20 hpi. Hence, it was suggested that *F. columnare* could pass through the membrane of zebrafish embryos and that the head part of the embryo was more susceptible to *F. columnare*.

To provide foundation for the development of a recombinant subunit vaccine, five membrane-associated proteases were analyzed in this study. The antigenic regions of five membrane-associated proteases, i.e. zinc metalloprotease, prolyl oligopeptidase, thermolysin metallo-peptidase, collagenase and chondroitinase AC, were connected and expressed in *E. coli*. The expression products were purified and injected into adult zebrafish as a vaccine. Each zebrafish in test group was injected with 1 µg (10 µl) purified protein. Fish in control group was injected with 10 µl sterilized saline. The immunized fish were subsequently challenged with the virulent strain G_4 three weeks after immunization. Number of dead zebrafish was recorded during one week. As a result, a relative protective rate of 79% was obtained. The results indicated that the prokaryotic expression product of the antigenic regions of five membrane-associated proteases could be a candidate protective antigen for the prevention of columnaris disease at least in zebrafish.

To screen the protective antigen of *F. columnare*, total protein of G_4 was extracted and separated by 2-D electrophoresis. Fourteen proteins were identified using an immunoblotting approach in two-dimensional electrophoresis map gels with antibacterial sera [2] from grass carp, *Ctenopharyngodon idella* (Valenciennes), and then anti-grass carp-recombinant Ig (rIg)

polyclonal antibodies. These proteins were characterized conclusively by matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF/TOF MS). These 14 proteins are considered as immunogenic molecules of *F. columnare*, including chaperonins DnaK, GroEL and trigger factor, and translation elongation factor G, translation elongation factor Tu, 30S ribosomal subunit protein S1, dihydrolipoamide succinyltransferase, succinyl-CoA synthetase, SpoOJ regulator protein, alcohol dehydrogenase, fructose-bisphosphate aldolase, 3-hydroxybutyryl- CoAdehydrogenase and two conserved hypothetical proteins.

In conclusion, the infection with *F. columnare* may start as early as from eggs for zebrafish. Antigenic regions of five protease genes were fused and expressed in *E. coli*, and the fused protein was confirmed to have some protection against columnaris disease in zebrafish. Besides, fourteen immunogenic proteins of G_4 were isolated, which may be possible candidate genes for vaccine development.

References

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