

Cloning of ORFs of Rock Bream Iridovirus (RBIV) for the Development of Vaccine Against RBIV

Yun-Im Kim, Seo, Hyo-Jin¹, Jung-Hyun Lee, Ki-Hong Kim²,
Yoon-Kwon Nam³, Sung-Koo Kim

Department of Biotechnology and Bioengineering, Pukyong National University.

¹Interdisciplinary Program of Marine Biotechnology, Pukyong National University.

²Department of Aquatic Life Medicine, Pukyong National University.

³Department of Aquaculture, Pukyong National University

Iridoviruses are well known as causative agents of serious systemic diseases in many fish species. The iridoviral diseases with high mortalities have been reported in worldwide. Recently, complete genomic DNA sequence of RBIV was reported(1), however, the antigenic genes of this virus were not yet known.

In this study, 3 ORFs of RBIV related with viral attachment or infection were cloned. These ORFs of RBIV were cloned into pGEX 4T-1 vector containing GST gene for the expression and the purification of recombinant proteins(2).

After the transformation of the recombinant plasmids into *E.coli* BL21(DE3), the cells were cultured with IPTG induction. The expressed proteins with 4h culture after IPTG induction were detected by SDS-PAGE analysis with 12.5% acrylamide gel.

The condition of the protein over-expression was optimized by the cell culture with various concentrations of IPTG, culture temperatures and induction times. The recombinant proteins expressed in the optimized condition were purified with GST affinity chromatography.

The purified protein was injected into BALB/c mice (4 mice/one group, 6 to 7 weeks old) to obtain the polyclonal antibody. Four booster injections to all groups of the mice were carried out 1 week intervals. At 7 days after final injection, sarcoma cells containing 1×10^7 cells was injected intraperitoneally to all

group do mice.

The produced polyclonal antibody was used for the western blotting to identify the immune-reaction ability.

References

1. Jeong Wan Do, Chang Hoon Moon, Hyo Jeong Kim, and Jeong Woo Park, *et al.*, "Complete genomic DNA sequence of rock bream iridovirus"(2004), *Virology*, Vol. 325, 351-363.
2. GST gene fusion system (1997), third edition, revision 2, Amersham pharmacia biotech, Inc.