Overexpression of Glutathione S-transferase Fusion Hirame Rhabdovirus Coat Protein as Inclusion body and Its Purification

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Summary

Hirame rhabdovirus (HIRRV) is one of the fish pathogenic virus and the viral glycoprotein (G) is a spike protein which spans the viral envelope and protrudes toward the exterior of virion. In this study, C-terminal half of G protein was expressed as about 55kDa glutathione S-transferase (GST) fusion protein. The expression of GST-G fusion protein from *E. coli* BL21(DE3)/pGEXHRG' was induced by various concentration of IPTG at OD_{600} = 0.5 with fermentation. The cells were sonicated and washed with lysis buffer, and the inclusion bodies were denaturated by 8M urea and refolded by simple dilution with 10mM Tris-HCl (pH 8.0) buffer. The fusion protein was purified by ion exchange chromatography (DEAE Sephadex A-25) and then it was purified by affinity chromatography using glutathione agarose. The bound proteins were eluted by elution buffer containing various concentration of reduced glutathione. Finally, for concentrating the eluted GST-G protein solution, ultrafiltration was used and 4.47mg/ml of purified GST-G protein was obtained. The fusion protein was digested with thrombin protease and the result was analysed by SDS-PAGE.

References

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