Production and purification of the phosphoprotein of Nipah virus in Escherichia coli for use in diagnostic assays.

Abstract

ABSTRACT Nipah Virus (NiV) is an emerging zoonotic paramyxovirus that can be fatal in humans and various types of animals. The phospho (P) protein of NiV plays an important role in RNA synthesis, replication, and genome synthesis. In this study, the NiV P gene was cloned into a pTrcHis2-TOPO vector and the recombinant protein containing a His-tag was produced in Escherichia coli. SDS-PAGE and Western blot analysis using the anti-His antibody confirmed the protein expression. An optimization study of E. coli fermentation showed that the optimal cultivation temperature was 37Â°C, while the optimal induction time for P protein expression was at 9 h with 1 mM IPTG. Solubility analysis showed that E. coli cultivated at 37Â°C produced the highest fraction (70%) of soluble P protein. The recombinant P protein was purified from clarified E. coli lysate using an immobilized metal affinity chromatography (IMAC) technique to a purity of 92.67%, with a purification factor of 11.58. The purified P protein strongly reacted with the anti-NiV swine sera collected during a NiV outbreak, suggesting its potential as a diagnostic reagent.

Keyword: Nipah virus; Phosphoprotein; IMAC; ELISA; Antigenicity; Diagnostic agent.