Molecular Cloning And Expression Analysis Of The Plpb Protein From Pasteurella Multocida A:1 (PG0013)

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Abstract

\textit{Pasteurella multocida} is a Gram-negative bacterial pathogen known to affect a wide range of domestic and wild animals. The increasing incidence of \textit{P. multocida} isolation from cases of fowl cholera and hemorrhagic septicemia in most part of the world has led to a renewed interest in this pathogen as well as in the development of vaccines. The currently available vaccines, bacterins and modified live vaccines, have limited efficacy. This led us to consider subunit vaccines based on individual antigens of \textit{P. multocida}. In this study, the PCR primers were designed based on the nucleotide sequence of \textit{P. multocida}. Pm 70 which was already published and used to amplify the fragment of \textit{plp B} gene from \textit{P. multocida} A:1. The \textit{plp B} gene, consisting of 831 base pairs with a predicted molecular weight of 39 kDa, was cloned and expressed. The purified PCR product was inserted into the plasmid carrying a gene encoding for GST protein. The expression of recombinant GST-tagged PlpB protein in \textit{Escherichia coli} was detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and purified by affinity column chromatography. The purified GST-fusion recombinant protein showed a single band migrated at about 70 kDa on SDS-PAGE gel which was an approximate size of GST tag and the PlpB protein. After cleavage by the GST protease, the separated protein was presented at about the same size as the predicted PlpB protein. In summary, we report that the \textit{plp B} gene was successfully cloned under the unnatural promoter and host. The recombinant PlpB protein is under the process of characterization and determination for possibility to be a subunit vaccine against \textit{Pasteurella multocida} infection.

Key words: Pasteurella multocida, Fowl cholera, PlpB protein, Recombinant protein, Subunit vaccine

Introduction

The Gram negative bacterium Pasteurella multocida is the etiological agent of hemorrhagic septicemia in cattle, atrophic rhinitis in pigs and fowl cholera in birds. \textit{P. multocida} strains can be separated into serogroup A, B, D, E and F based on the antigenicity of their capsules and serotype 1 to 16 based on lipopolysaccharide antigens. The capsular serogroup is generally related to diseases with the majority of fowl cholera strains belonging to serogroup A. Fowl cholera is a common avian disease, which may affect all types of birds throughout the world. Capsulated strains of \textit{P. multocida} serovar A and somatic serotypes 1, 3 and 4 are recognized as the primary cause of fowl cholera. The increasing incidence of \textit{P. multocida} isolation from cases of hemorrhagic septicemia and fowl cholera in most parts of the world has led to renewed interest in this pathogen as well as in the development of vaccines for prevention of these infections.

Current \textit{P. multocida} vaccines contain either inactivated bacteria or live attenuated bacteria. The inactivated vaccines induce only serotype-specific immunity. Attenuated live vaccines can provide limited heterologous protection (Rimler and Rhodeas, 1981) but sometimes induce the disease (Bicrer and Derieux, 1973; Carpenter et al., 1988; Prantner et al., 1990; Schlink and Olson, 1987). Recently, the development of subunit vaccines comprising of purified subunits from this microorganism has been promoted. Previous study have shown that outer membrane protein preparations from \textit{in vivo} grown \textit{P. multocida} were able to protect birds from challenge with heterologous serotype while \textit{in vitro} grown bacteria were able to protect only against the homologous serotype (Glisson and Cheng, 1991; Heddleston et al., 1970; Heddleston and Rebers, 1972). \textit{In vivo} expressed proteins responsible for heterologous protection have been termed cross-protective factors (Rimler, 2001). Recently, the 39-kDa antigen was purified by SDS-PAGE and electrophoresion from hydroxyapatite chromatographic fractions of both \textit{in vivo} and \textit{in vitro} grown \textit{P. multocida}. The purified antigen can induced