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Expression of recombinant *Lawsonia intracelluaris* proteins

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Introduction

Lawsonia intracellularis (LI) is an obligate intracellular bacterium and the causative agent of proliferative enteropathy (PE) in pigs as well as a variety of other species. Characterization of the gene products from this microorganism may assist studies to identify the mechanism that influences the progression of infection and identify proteins for use in sero-diagnosis of PE. The objective of this study was to clone, express and purify recombinant proteins from 10 LI genes into *E. coli* for use in sero-diagnosis of PE.

Materials and Methods

1. Cloning of genes and construction of destination vectors

LI, strain PHE/MN1-00, served as the source of template for all PCR amplifications. Primers were designed from each coding sequence and contained sites for recombinational cloning into the Gateway Entry vector pDONR221. Each attB-flanked PCR product was cloned into an attP-pDONR221 vector using Gateway cloning technology to create a BP site. The BP reaction products were transformed into DH5α. Analysis of positive entry clones was performed by PCR and sequencing using M13 primers. Plasmids from the entry clones were subcloned into pDEST17 vectors to create destination plasmids by using Gateway LR recombinational cloning. The LR reaction products were transformed into DH5α. The destination clones were selected and analyzed with PCR and sequencing using T7 primers.

2. Expression and purification of recombinant proteins

Destination plasmids were purified and transformed into BL21-AI competent cells. Recombinant proteins (with 6His-tags) were overexpressed by induction with 0.2% L-arabinose and purified by Ni-NTA affinity chromatography.

3. Western immunoblotting

Proteins were electrophoretically transferred onto PVDF membranes with a tank transfer system. After transfer of proteins to the PVDF membrane, they were probed with polyclonal LI rabbit antibody (1:1000) or swine exposed LI sera (1:100). Goat anti-rabbit IgG–ALP conjugate (1:1000) or goat anti-pig IgG-ALP conjugate (1:1000) was used as the secondary antibody. The blot was developed with 3,3'-Diaminobenzidine (DAB) substrate.

Results

1. Cloning, expression, and purification of LI sequences in *E. coli*

Ten candidate genes were successfully cloned into bacterial expression vectors, expressed and purified. Coomassie-stained SDS–PAGE analysis revealed protein samples carrying insoluble recombinant proteins after induction by 0.2%L-arabinose.

2. Western immunoblotting

Western blots show that some of the expressed proteins, including two flagellar proteins, one hemolysin protein, one outer membrane protein and two other functional proteins immuno-reacted with both of the polyclonal rabbit antibody against whole cell LI and swine exposed LI sera. However, some recombinant proteins reacted slightly with normal swine sera.

Conclusions

This study shows that the availability of the genomic sequence of LI enables the identification of potential targets for the rational development of proteins for pathogenesis studies and antigens for sero-diagnosis of PE.