Cloning, Over-Expression and Purification of inlB Gene/Protein From *Listeria monocytogenes* for Possible Use in Drug Delivery

Wayne State College

Brittany Cody, Derek Moormeier, Doug Christensen, Shawn Pearcy, Wayne State College, Wayne NE 68787

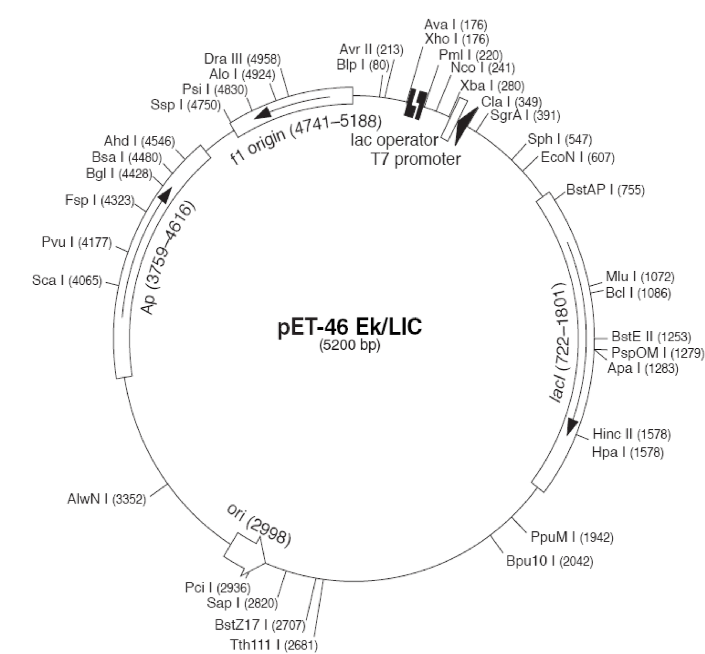
Introduction

Listeria monocytogenes is a food-borne pathogen with an exceptional ability to invade mammalian host cells that would normally not internalize the bacterium. There are several vital proteins that allow *L. monocytogenes* to induce phagocytosis in host cells and rapidly spread throughout host tissues. Internalin proteins, including inlB, bind to the cell membranes of typically non-phagocytic host cells causing the host cells to internalize the bacterium (Gaillard et al, 1991; Dramsi et al, 1995).

The unique ability of *L. monocytogenes* to cause phagocytosis within host cells is of interest as it may be the key to creating an alternative method of drug delivery. Oral ingestion is the most common method of drug delivery because of its simplicity and ease. However, this method also has several potential drawbacks including breakdown of the drug within the digestive tract, inefficient absorption of the drug, and inability of the drug to selectively target desired cells and tissues. This could lead to a decrease in the effectiveness of the drugs or require that they be taken at such high dosages that they become toxic to many cells and tissues within the body. A direct uptake of a drug via phagocytosis may bypass many of these obstacles.

The goal of this project is to produce and isolate the inlB protein in order to assess its potential as part of a drug-delivery module.

Materials & Methods

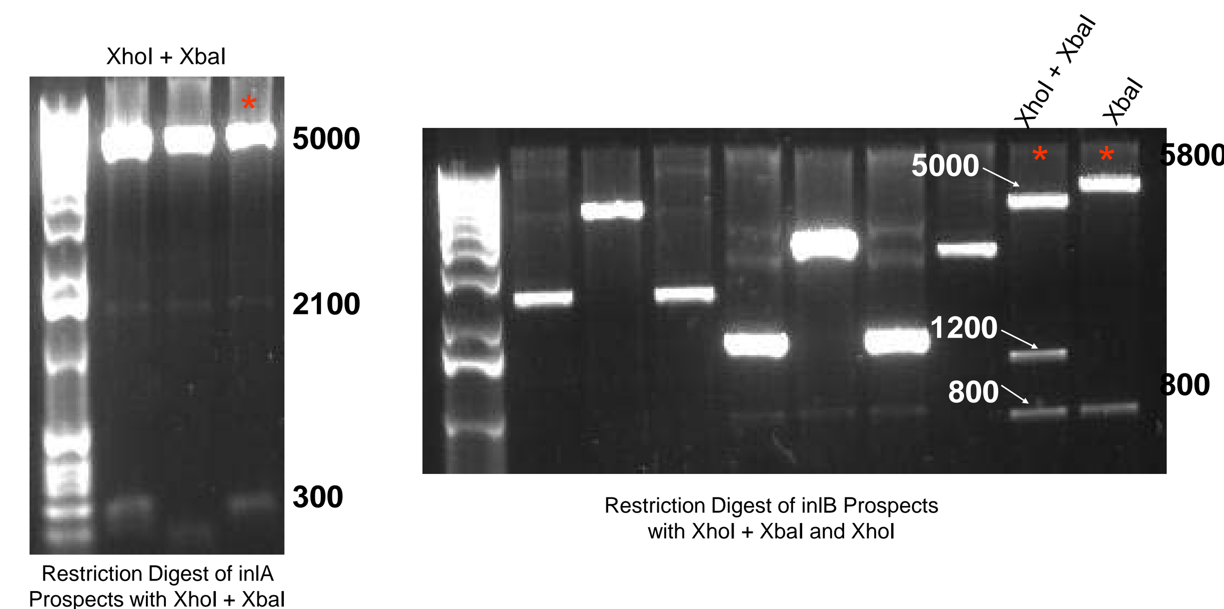


Cloning and Upregulation of inlB

The inlA and InlB genes were cloned into both NovaBlue GigaSingles™ Competent E. coli cells (Novagen) and C41 SOLOS Chemically Competent E. coli cells (Lucigen) using Ek/LIC pET-44 and Ek/LIC pet-46 cloning vectors. Both vectors incorporate a six histidine tag for later purification of the protein and the pET-44 vector contains a "Nus" region designed to increase cytoplasmic solubility. In both vectors gene regulation is controlled by a lac operator allowing for upregulation of the gene through the use of IPTG. The vector also incorporates a gene for ampicillin resistance allowing for the selection of vector-containing cells in media. After transformation, cells were streaked onto agarose plates with 10 mM ampicillin. Suspected insert-containing colonies were grown in LB broth containing 10 mM ampicillin.

Confirming Plasmid Transformation

- After growing prospective colonies in LB broth, plasmid isolations were performed (Zyppy Plasmid Miniprep, Zymo Research). The isolates were subjected to restriction digests with the enzymes SphI and XhoI and seperated in a 1% agarose gel. Those exhibiting expected digest patterns were used for protein isolations.
- Suspected insert-containing plasmid of the pET-46 vector inlB was sent to a core facility to be sequenced (UNMC-James Eudy, Director).



Restriction Digest of inlA Prospects with XhoI + XbaI

Restriction Digest of inlB Prospects with XhoI + XbaI and XhoI

Protein Isolation

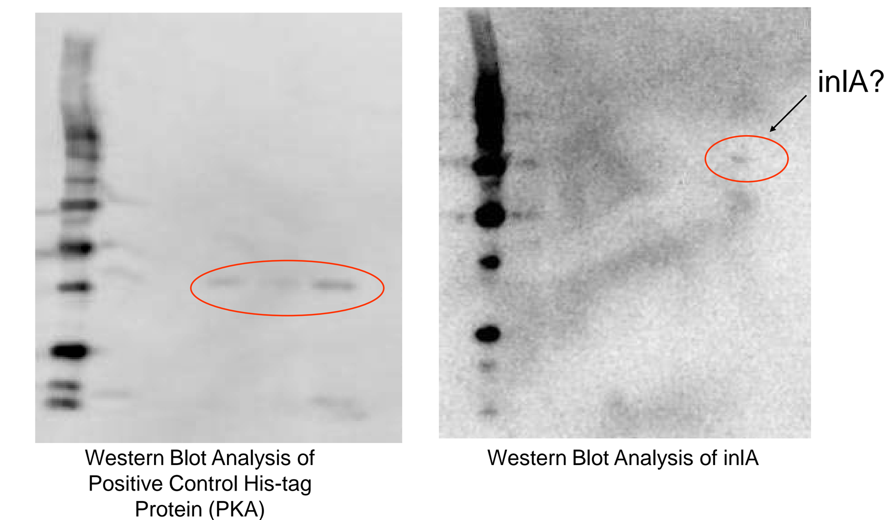
Cells were spun down, then resuspended, lysed, and sonicated.

****Dissociated membrane & checked (Triton® X-100, Sigma).

Cytoplasmic contents were then poured into spin columns specific for histidine-rich polypeptide regions. Untagged proteins were eluted out in a series of washes. His-tagged proteins were then eluted out to be used for Western Analysis (Ni-NTA Spin Kit, Qiagen).

Western Analysis

Elutions collected from histidine-specific spin columns were run on standard mini-gels (BioRad 10% Tris-HCL) along with supernatant. Proteins from the gel were transferred to a membrane using a semidry technique. The membranes were blocked in PBS blotto with 5% NFD milk. The membranes were later probed with rabbit polyclonal igG primary antibodies specific for 6-histidine tags and goat anti-rabbit secondary antibodies (Santa Cruz Biotech) with HRP chemiluminesence. A positive control using a His-tagged PKA protein was successful. The protein appears to be present in both spin column elutions as well as from supernatant. Westerns of inlA have been somewhat successful. Although we have not isolated inlA from spin column elutions, it appears to be present in supernatant. We have not been able to isolate inlA from either spin column elutions or supernatant.



Western Blot Analysis of Positive Control His-tag Protein (PKA)

Western Blot Analysis of inlA

Discussion

Restriction digests of inlA prospects were promising and showed expected band patterns after undergoing a restriction digest. We are still awaiting sequencing results for the gene, but Western blots of the inlA protein using the media supernatant in which inlA-containing inserts were grown appear to be successful.

Restriction digests of inlB prospects were also promising, resulting in expected digest patterns, so we attempted to isolate the inlB protein. After several failed attempts, we sent an inlB to the UNMC core lab (James Eudy) for sequencing. The results indicated that the plasmid insert did contain the inlB gene, that the promoter region was free from mutations, that the gene had no insertions or deletions that may have changed the reading frame, and that both the beginning and end of the gene had no mutations. We are currently working on sequencing the middle region of the inlB gene to ensure that there are no mutations present and working on new methods of isolating the protein. Part of the difficulty may result from inlB being a membrane protein. We have tried using Triton X100 detergent to help extract the protein from the membrane, but our efforts have thus far proved unsuccessful.

IDEAS??

The next step will be to expose HeLa cell cultures to the isolated proteins. Western analysis will be done to analyze the ability of the proteins to initiate internalization by the cells. If internalization is observed, we will attempt to bind the protein to an inert carrier and again measure internalization as this may indicate the potential of the protein as a drug delivery module.

References

•Dramsi S, Biswas I, Maguin E, Braun L, Mastroeni P, Cossart P. (1995) Entry of Listeria monocytogenes intohepatocytes requires expression of InlB, a surface protein of the internalin multigene family. Mol Microbiol 16, 251–261.

•Gaillard JL, Berche P, Frehel C, Gouin E, Cossart P., J.L. (1991) Entry of L. monocytogenes into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from Gram-positive cocci. Cell 65, 1127–1141.

This work was supported by NIH Grant Number 1 P20 RR16469 from the INBRE Program of the National Center for Research Resources.