Characterization of Novel Binding Interactions of E. coli O157:H7 in Plant Cells

Jasmine Moore and Lindsay Gielda, PhD
Department of Biology and Chemistry, Purdue University North Central, Westville, IN

Introduction

- Enterohemorrhagic *E. coli* (EHEC) infections cause severe gastrointestinal illness in humans and are acquired through consumption of fecal-contaminated food products
- EspB is a secreted protein by the type III secretion system (TTSS) used by *E. coli i*n infection
    - TTSS is a needle-like appendage that detects eukaryotic cells and secretes effector proteins to aid in bacterial infection
- EHEC enters the gastrointestinal tract and secrete toxins into the host endothelial cells and bloodstream
- EspB is secreted into the host cell and has an important role in altering host cell signal transduction pathways.
- Hypothesized that a similar mechanism is utilized in EHEC binding to plant tissue.
- Purpose of study was to express a Histidine tagged-EspB protein in a nonpathogenic *E. coli* strain

Methods

- Cloned EspB with both a 5’ and 3’ 6x-His-tag onto the pUC19 vector in E. coli strain BL21 (Fig. 1)
    - Attached His-tag both ways to determine greatest yield
- After verification of successful cloning, expressed EspB gene
- Western Blot analysis on whole cell lysates
    - Protein purification using affinity beads was done to isolate the proteins

References

- Mathews, S. L., Smith, R. B., & Matthysse, A. G. (2014). *Microbial Biotechnology, 7*(6), 570-579
- Matthysse, A. G., Deora, R., Mishra, M., & Torres, A. G. (2008). *Applied and Environmental Microbiology, 74*(8), 2384-2390.

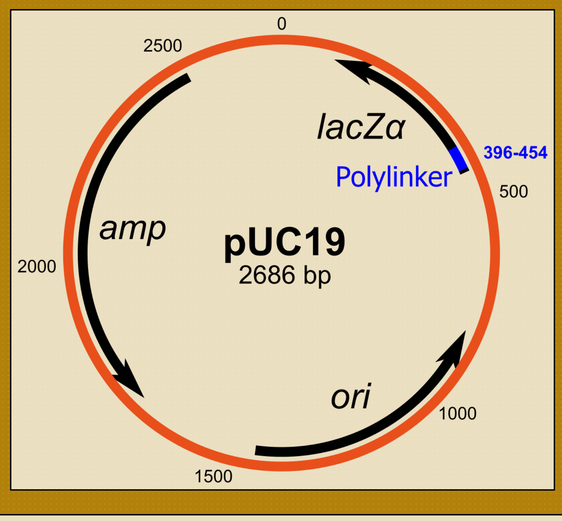

Fig. 1 pUC19 vector used
Genes for *amp* and *lacZ* allow for differentiation of successfully cloned cells

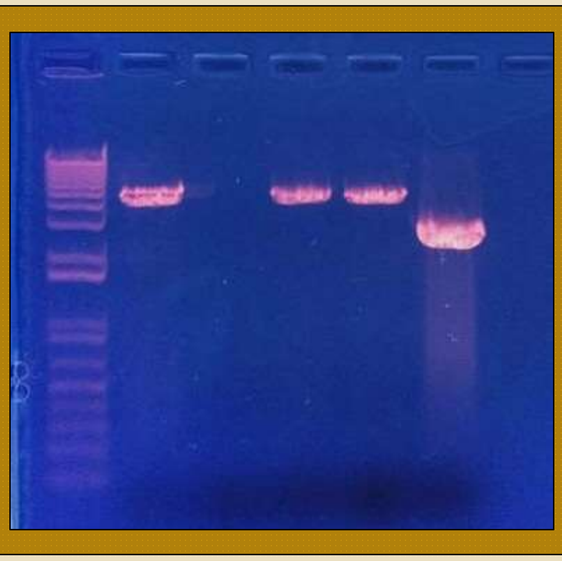

Fig. 2 Expression of EspB insertion in Puc19 vector
Lane 1: DNA Ladder
Lanes 2 and 3: 5’ His tagged EspB
Lanes 4 and 5: 3’ His tagged EspB
Lane 6: pUC19 vector

Fig. 3 Blot of EspB purification of His-tagged cell lysates
Lane 1 (bottom): Protein ladder
Lane 2: 5’ His tagged EspB
Lane 3: 3’ His tagged EspB

Results

- Successfully cloned EspB 6x His-Tag in BL21 *E.coli* strain
    - Shown by results of cloning and gel electrophoresis (Fig. 2)
    - Expression of His-tagged EspB was successful for both 5’ and 3’ directions
- A pull down assay on whole cell lysates purified the EspB protein
    - Conducted with nickel affinity beads
- Western blot analysis confirmed the production of the tagged protein (Fig. 3)

Discussion

- Both EspB and TTSS are necessary for *E. coli* attachment to human epithelial cells and subsequent infection
- Isolated proteins will be used in future experiments to identify plant binding partners to EspB
- Development of procedures to better expose His-tagged EspB
    - Result in stronger western blot results
- EHEC is capable of entering human endothelial cells with the Gb3 receptor, which allows effector proteins to enter the tissues
- If produce has a similar receptor, EHEC may use the same mechanism for binding to plant surfaces
- Studying the molecular interactions between EHEC and its plant host, may lead to the development of targeted treatments to limit the transmission of EHEC to humans

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