Development of an Expression Vector for the Internalin A Domain from Full-Length Internalin, a Key Invasion Protein of *Listeria monocytogenes*

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Abstract

Listeria monocytogenes is a foodborne pathogen capable of invading intestinal epithelial cells through interactions between its surface protein, Internalin A, and the host receptor E-cadherin. This project began with the goal of expressing full-length Internalin A (InlA') to support studies on host cell invasion. While initial efforts focused on vector development, the project evolved into optimizing PCR-based methods for amplification and purification of InlA' DNA. A reliable workflow was established, laying the foundation for future cloning and expression studies.

Introduction

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive, foodborne pathogen and the causative agent of listeriosis, a rare but life-threatening infection with high mortality rates. Known for its ability to survive harsh environmental conditions—such as extreme temperatures, acidic and saline environments, and aggressive cleaning procedures—L. monocytogenes is particularly resilient. These survival mechanisms enable it to persist in food processing environments and contribute to its successful invasion of intestinal epithelial cells, a critical step in its pathogenicity. Understanding how it survives and invades host cells is essential for developing targeted therapies.

A key factor in *L. monocytogenes'* pathogenesis is its ability to invade non-phagocytic epithelial cells of the intestinal barrier. This process is primarily mediated by a surface protein called Internalin (InlA'), which facilitates entry by binding to E-cadherin (hEC1), a host adhesion molecule found on epithelial cells. This interaction mimics natural cell-cell adhesion, tricking the host into responding as if to a neighboring cell. The resulting intracellular signaling triggers cytoskeletal rearrangements that allow L. monocytogenes to be internalized, initiating infection.

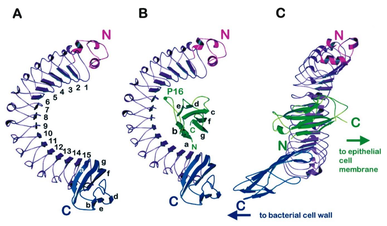


Figure 1 Structure of InlA'. (A) Uncomplexed InlA': cap domain-pink, LRR-domain-violet, Ig-like interrepeat domain-blue; (B) InlA'/hEC1 complex viewed as in (A) and (C) rotated by 90°: hEC1-green.

Internalin A is therefore a critical determinant of host specificity and tissue tropism, making it a valuable target for understanding how *L. monocytogenes* breaches the intestinal barrier. Studying these molecular interactions not only provides insight into bacterial invasion strategies but may also inform the development of therapies aimed at preventing or limiting infection.

Materials and Methods

Polymerase Chain Reaction (PCR) is a technique used to rapidly and specifically amplify DNA. It was used to generate the InlA' gene for downstream cloning and expression.

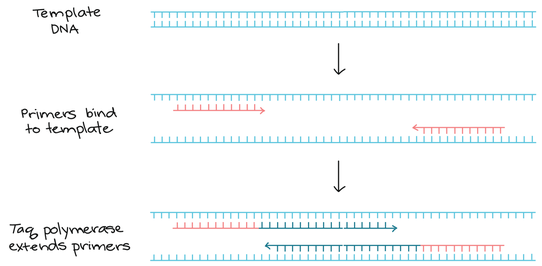


Figure 2 Primer Binding & DNA synthesis in PCR. Primers bind to single-stranded template DNA after denaturation. DNA polymerase (Taq polymerase) extends each primer, synthesizing new DNA strands complementary to the target sequence.

Technical challenges in obtaining the correct DNA shifted the focus to optimizing PCR conditions. Multiple rounds were performed to refine primer design, confirm full-length products, and produce high-quality DNA for purification. Developing a reliable PCR workflow became a key outcome of this project. All PCR samples were verified via Agarose Gel Electrophoresis.

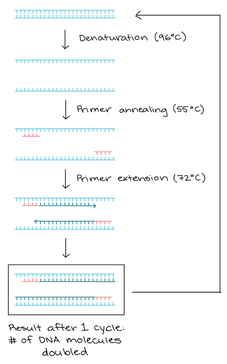


Figure 3 PCR Cycles. Illustration shows each step throughout a single PCR cycle, including denaturation, annealing of primers, and extension by DNA polymerase. The temperatures and timing for each step were optimized to ensure efficient amplification of InlA'.

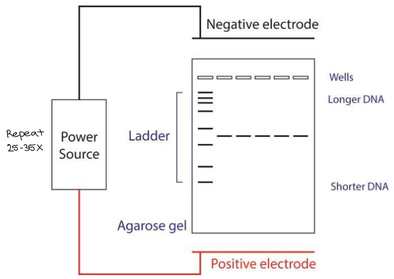


Figure 4 Gel Electrophoresis. Illustration of how gel electrophoresis separates DNA fragments based on size. Negatively charged DNA is pulled through the gel by an electric current toward the positive electrode. Smaller fragments move more quickly through the matrix, allowing for size-based separation. A DNA ladder is included as a molecular weight reference. Single, prominent bands suggest successful and specific target DNA amplification.

Table 1. Designed Primers for FL Internalin and Active InlA' Domain

Name	Sequence
InlA'5x	GCG CCA TAT GGC TAC AAT TAC ACA AGA TAC
InlA'3x	GCG CGG ATC CTT ACC CAG CTT CCA CTT C
FLInlA5x	GCG CCA TAT GAG AAA AAA ACG ATA TGT AT
FLInlA3x	GCG CGG ATC CTT ACT ATT TAC TAG CAC GTG CT

Table 2. Initial and Final PCR Reaction Conditions for Amplification of InlA'

	Initial PCR Mixture	Final PCR Mixture
Method	TaqDog Premium 2x Green Master Mix	NEB One*Taq* Quick Load 2x Master Mix
Mix	18µL Nuclease-Free H_2O 25µL Buffer (2x green master mix) 2µL template DNA 2.5µL 5x Primer 2.5µL 3x Primer	8µL Nuclease-Free H_2O 25µL Buffer (OneTaq 10x GC Buffer) 2µL Template DNA 2.5µL 5x Primer 2.5µL 3x Primer 10µL TaqDog PCR enhancer

Results

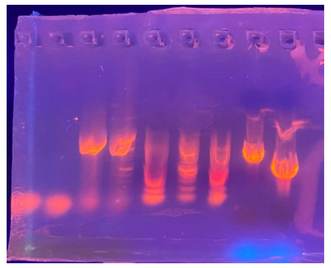


Figure 5 Initial PCR reactions Agarose Gel. Lanes from left to right: FL #1, FL #2, InlA' #1, InlA' #2, Old ladder, new ladder (6µL), new ladder (10µL), S. pace FL, BFP. Smeared bands indicate potential non-specific amplification, DNA degradation, or the presence of many different sized DNA fragments. Variation in band intensity suggests different concentrations of DNA within the original samples.

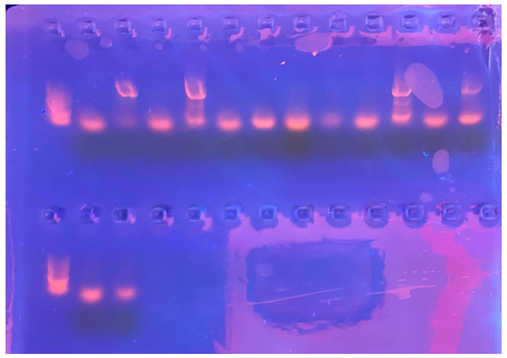
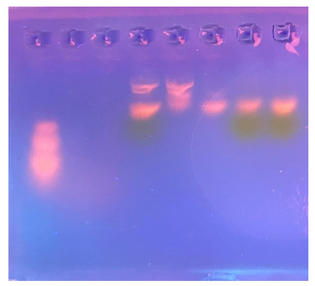


Figure 6 All Altered PCR Reactions Gel. Lanes from left to right: ladder, 55°C FL + 10µL enhancer, 55°C InlA' + 10µL enhancer, 52°C FL NO enhancer, 52°C InlA' NO enhancer, 52°C FL + 10µL enhancer, 52°C + 10µL enhancer InlA, 52°C FL + 18µL enhancer, 52°C InlA' + 18µL enhancer, 48°C FL NO enhancer, 48°C InlA' NO enhancer, 48°C + 10µL enhancer FL, 48°C + 10µL enhancer InlA', 48°C + 18µL enhancer FL, 48°C + 18µL enhancer InlA'

Figure 7 New PCR Protocol Gel. Lanes from left to right: ladder, 48°C FL TaqDog, 48°C InlA' TaqDog, 55°C + 10µL InlA' TaqDog, 55°C + 10µL enhancer InlA' New England Bio (NEB) Labs, 55°C + 10µL FL NEB, Hybrid #1 (5x FL primer, 3x InlA' primer), Hybrid #2 (5x InlA' primer, 3x FL primer).



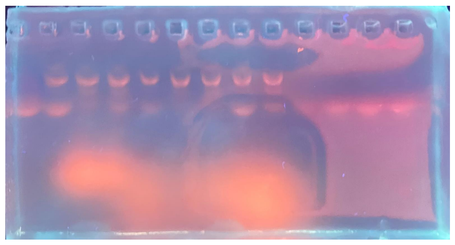


Figure 8 NEB + enhancer PCR Gel. Lanes from left to right: Ladder, lanes 2-8 InlA' PCR mix, lanes 9-12 FL PCR mix. Though there are consistent, larger DNA fragments present in lanes 2-8, the significant smearing at the bottom suggests the DNA quality or specificity of the reaction was compromised. In response, a lower voltage (50 V) for longer periods of time (~20 min) was used for future PCR samples..

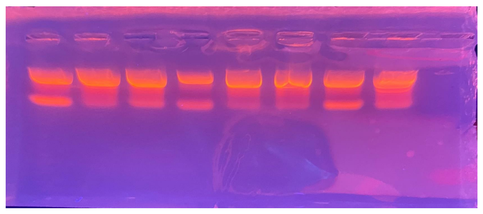


Figure 9 Final Eight InlA' PCR Samples Gel. All eight lanes show identical PCR samples, with consistent band size and intensity, indicating successful amplification of InlA'. The additional faint bands observed below the main bands suggest the presence of non-specific PCR products or primer dimers. These secondary bands are removed during gel extraction and DNA purification.

Conclusion

- Completed all cloning steps intended to insert InlA' domain into pET28a(+) vector using NedI and BamHI restriction sites
- Transformation into DH5-a E. coli cells was unsuccessful
- Identified reliable methodology for amplification and purification of InlA'
- Compiled a detailed workflow to make cloning and transformation processes more accessible for future students

Future Directions

- Repeat transformation process using new, high-efficiency competent *E. coli* cells
- Verify insertion of InlA' gene using colony PCR and sequencing
- Develop expression and purification protocol for InlA' and FL Internalin using the His-tag system
- Study protein interactions using biochemical assays to advance research of pathogen-host interactions and production of targeted Listeriosis therapies

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