Confirmation of Transposon Positions in the Bacterium Proteus mirabilis HI4230 Mutants

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Confirmation of Transposon Positions in the Bacterium *Proteus mirabilis* HI4230 Mutants

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Abstract

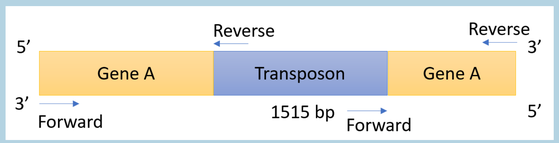
Biofilms, complex microbial communities encased in a self-produced extracellular matrix, play a vital role in chronic infections and antibiotic resistance. Several disease-causing bacteria, like *E. coli* and *Proteus mirabilis*, form robust biofilms during infections. This study investigates the positions of transposons in the bacterium *Proteus mirabilis* HI4230 which will reveal specific genes that may contribute to biofilm formation. First attempts to identify the transposon positions were not successful. Several factors may have played a role including primer design and reagent quality.

Background

- *Proteus mirabilis* is a motile, Gram-negative facultative anaerobic bacterium commonly found in the intestinal tracts of various animals and humans (Jacobsen et al., 2008)
- *P. mirabilis* is an opportunistic pathogen that causes urinary tract infections (UTIs), pyelonephritis, bacteremia, respiratory tract infections, meningitis, and biofilm development on medical equipment (Drzewiecka, 2016)
- Biofilms are complex formations of microorganisms that are encased in an extracellular matrix which provides protection, increased antibiotic resistance, and supportive resource sharing
- Transposons alter gene expression allowing researchers to study the function of genes

- The focus of our research is to determine if particular genes affect the biofilm formation of *P. mirabilis*
- The genes analyzed in this study were the *dmsA* gene which encodes for dimethyl sulfoxide reductase chain A protein and the *cspC* gene which encodes for a cold shock-like protein
- First, we need to confirm the location of transposons in *P. mirabilis* mutant chromosomes before any analysis can be done

- **Basic lab design for the confirmation of a transposon location**: To confirm the location of the transposon, we needed to design forward and reverse primers to amplify the region where the transposon is thought to be located using PCR (Image below). The PCR products are then visualized using agarose gel electrophoresis. These PCR products need to be sequenced to confirm the transposon locations by isolating the products and cloning the DNA fragments in *E. coli* using a plasmid.



Overview of an Example Gene, Transposon, and Primer Locations

Purpose

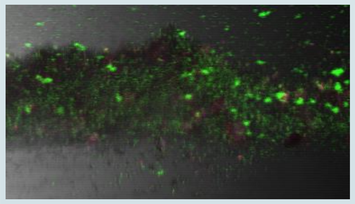
To determine which genes and their products affect biofilm formation in the bacterium *Proteus mirabilis* by using transposon mutants

Materials and Methods

- **Bacterial Strains Used**: Urinary tract pathogen *Proteus mirabilis* HI4320 (wild type), HI4320 mutants were created using transposition using the mariner *Himar1* transposon and donated by the Mobley lab at University of Michigan (Pearson et al., 2022)
- **Culture Conditions:** *P. mirabilis* HI4320 was cultured by inoculated sterile Luria broth agar (LB, Fisher) with tetracycline (15 μg/ml, GoldBio). HI4320 mutants were inoculated in sterile LB with kanamycin (50 μg/ml, GoldBio). All cultures were grown statically at 37°C.
- **PCR**: Colony PCR procedures were followed using *P. mirabilis* HI4320 and mutant colonies for the templates and Taq polymerase (50 μl Total reaction, NEB). Melting temperatures (Tm) for the PCR primers were calculated using the following equation: Tm = 4 (GCs) + 2 (ATs)
- **Gel electrophoresis**: Loading buffer (purple dye, NEB) was added to the PCR fragments (10 μl final volume). The samples were loaded on a 1% agarose gel in TAE buffer and ran at 150 Volts. After electrophoresis, the samples were stained with SYBR Green (Invitrogen). A 100 bp and 1kb DNA ladder in loading buffer were used as the standards (10 μl final volume, NEB).

P. mirabilis Mutants and Transposon

Mutant	Gene Name	Size	Protein
PMI1705	*dmsA*	2439 bp	Dimethyl sulfoxide reductase chain A
PMI1676	*cspC*	213 bp	Cold shock-like protein
Other		**Size**	**Background**
Transposon Tn		1515 bp	*Himar 1* mariner transposon that only requires TA dinucleotide sequences for transposition

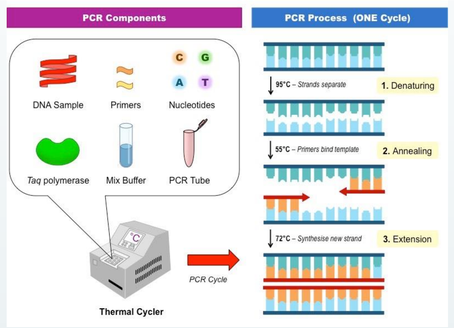


Proteus mirabilis biofilm formation on a glass flow cell when cultured in pooled human urine stained with a live dead stain, Confocal Laser Scanning Microscope

Experimental Set Up

For a standard 50 μL PCR reaction:

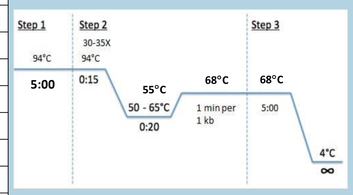
- 30 μl nuclease free water
- 5 μl 10x Taq Reaction buffer
- 1 μl 10 mM dNTPs
- 1 μl 100 μM stock Forward primer
- 1 μl 100 μM stock Reverse primer
- 1 *P. mirabilis* colony
- 2 μl diluted Taq DNA polymerase (0.5 units/20 μL PCR reaction, 1 μL Taq into 3 μL 1X reaction buffer)



Genes	Primers Sets Used in the Reactions
cspC	• Forward and Reverse primers for *cspC* gene • Forward and Tn primers for *cspC* gene • Tn and Reverse primers for *cspC* gene • Reaction with no Taq polymerase (negative control)
dmsA	• Forward and M2 primers for *dmsA* gene • Forward and Tn primers for *dmsA* gene • Tn and M2 primers for *dmsA* gene • M2 and Reverse primers for *dmsA* gene • Tn and Reverse primers for *dmsA* gene • Reaction with no Taq polymerase (negative control)

PCR Perimeters

Step	Temp (°C)	Time (min)
1	94	5:00
2	94	0:15
3	55	0:20
4	68	2:30
Repeat 30-35X		
5	68	5:00
6	4	∞

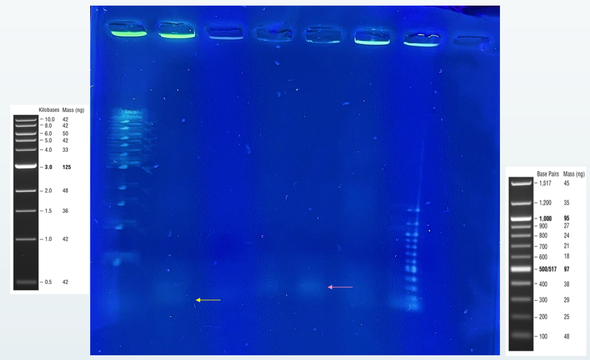


Primers Used

Gene	Forward Primer	Reverse Primer	Other Primers
cspC	5' ATGTCTGACAAAATGAAAGGT 3' (21 bp, Tm = 56°C)	5' TTACAGAGCTGTTACGTTAG 3' (20 bp, Tm = 58°C)	
dmsA	5' ATGTCAATGTCAAAAATTAAAAATA 3' (23 bp, Tm = 58°C)	5' TTATGCACGTTTCACTTCAAC 3' (20 bp, Tm = 54°C)	**M1**: 5' TGTAAGAGCCATTTTCAGGTG 3' (21 bp, Tm = 60°C) **M2**: 5' GATTGGTAACCACGATGCTG 3' (20 bp, Tm = 60°C)
Tn	5' CCAAGCAGAAGACGGCATA 3' (19 bp, Tm = 58°C)	Primer for the transposon is the same for the forward and reverse primers as the ends of the transposon have the same sequence	

Achilles The Bear, PTSD Service Dog & AACC Student Researcher

Results



Agarose Gel of the *cspC* PCR samples. The 10 μl samples were loaded on a 1 % TAE agarose gel and ran at 150 V. Well order from left to right: 1 kb ladder (NEB), Forward Tn *cspC,* Tn Reverse *cspC,* Forward Reverse *cspC* (Predicted to be 213 bp, pink arrow), No Taq Tn Reverse *cspC,* Forward Reverse *cspC,* 100 bp ladder (NEB), Low molecular band noted by a yellow arrow

Discussion

- There were low molecular weight bands for many of the PCR samples and the DNA ladders are visible. These bands could be the primers amplifying themselves. There is a faint band about 213 bp in the tube with the forward and reverse primers for the *cspC* gene using a wildtype *P. mirabilis* HI4320 colony (control)
- **Possible reasons for the results:** Primer design was flawed (Tm, dimer formation, etc.), Older reagents, Transposon insertion close to one end of the gene so a small product was formed, The transposon is in a different location

Future Research

- Confirm the sequences of the primers and check the Tms and hairpins
- Run the PCR again using newer reagents and run a Tm gradient
- Put the PCR fragments into a plasmid for sequencing
- More research on the roles of the genes in biofilm formation in *P. mirabilis* and other bacteria

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