Exploring *Proteus mirabilis* cyclic dinucleotide signaling network phenotypes during biofilm formation

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Abstract No. 4021

KEY FINDING

Transposon mutant screening identified candidates for biofilm inhibition in *Proteus mirabilis*.

INTRODUCTION

Urinary tract infections (UTIs) represent a significant healthcare burden affecting 150 million people annually. *Proteus mirabilis* is the causative agent of up to 44% of catheter-associated UTIs. With an estimated 48% of strains exhibiting antibiotic resistance, *P. mirabilis* has an arsenal of pathogenic strategies including swarming motility, urease production, and fimbria and biofilm formation. Biofilms are particularly relevant for clinical applications as they enhance antibiotic resistance and diminish host defense mechanisms leading to recurrent infections. Cyclic dinucleotides (CDNs) play a crucial role in biofilm formation by regulating the transition between motile and stationary lifestyles *via* production and secretion of adhesins, polysaccharides, and DNA, all of which contribute to the stability of the extracellular matrix. In this study, we explored the regulatory network of *P. mirabilis* HI4320 biofilm by utilizing BV-BRC and BioCyc databases to identify 14 genes with sequence similarity to enzymes involved in dinucleotide synthesis activity or its regulation. Investigating CDN regulation could lead to new strategies for disrupting biofilm formation and the prevention of antibiotic-resistant bacterial infections.

RESULTS

Biofilm Formation

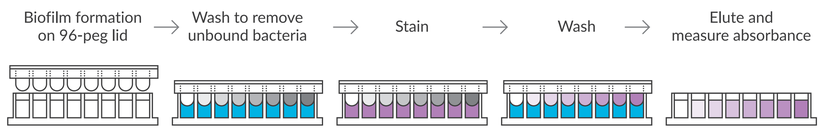

Figure 1 – Peg Lid-Based Biofilm Quantification Workflow.

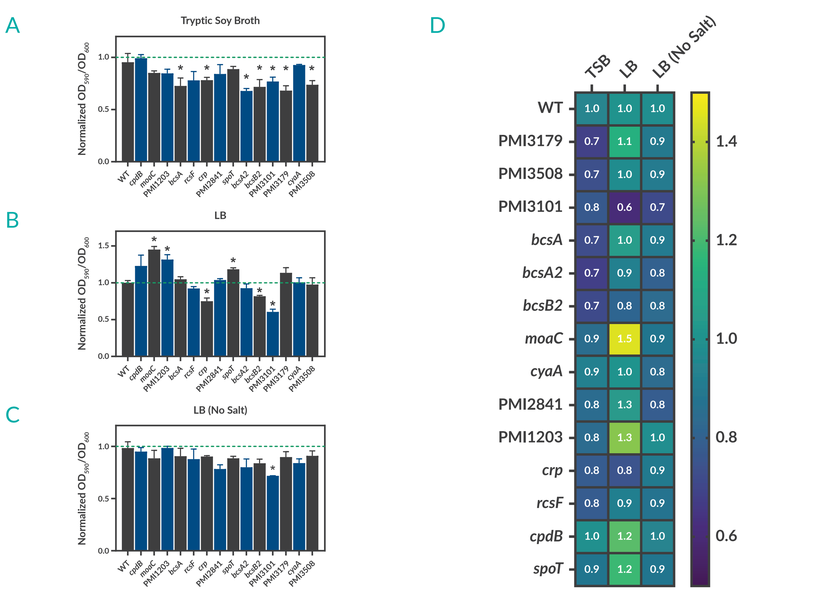

Figure 2 – A peg lid-based assay identified biofilm abnormalities.
(A-D) Overnight cultures of *P. mirabilis* transposon mutants were normalized and inoculated into the assay plate at a final OD_{600} of 0.05 in **(A)** Tryptic Soy Broth (TSB), **(B)** LB, and **(C)** LB without salt. Plates were incubated statically at 37°C for 24 hours. Absorbance at 590 nm was normalized to total growth (OD_{600}) and normalized to wild-type (WT). **(D)** A heat map showing the summary of results. *= p <0.05; N=3

RESULTS

Biofilm Formation - Continued

Using the peg lid-based biofilm quantification assay, several transposon mutants were determined to be defective in their ability to form biofilm under various conditions. The cultures were incubated statically at 37°C for 24 hours. Following incubation, the relative levels of biofilms formed on peg lids were determined. Pegs were washed and stained with crystal violet before measuring the absorbance at 590 nm. Transposon mutations that affected *moaC*, a gene encoding a putative phosphodiesterase, and PMI1203 both exhibited increased biofilm formation in LB compared to WT.

Quantification of Cyclic Dinucleotides *via* Competitive ELISA

Using competitive ELISA assay kits, the production of CDNs by *P. mirabilis* over 72 hours under biofilm forming conditions in either TSB or LB was quantified. Cyclic di-GMP production peaked in TSB at 72 hours whereas other CDNs assayed peaked significantly earlier. Transposon mutants PMI3508, *bcsA2*, PMI3101, *cpdB*, and *moaC* had increased CDN production. PMI2841 and *bcsA* had decreased CDN production compared to WT.

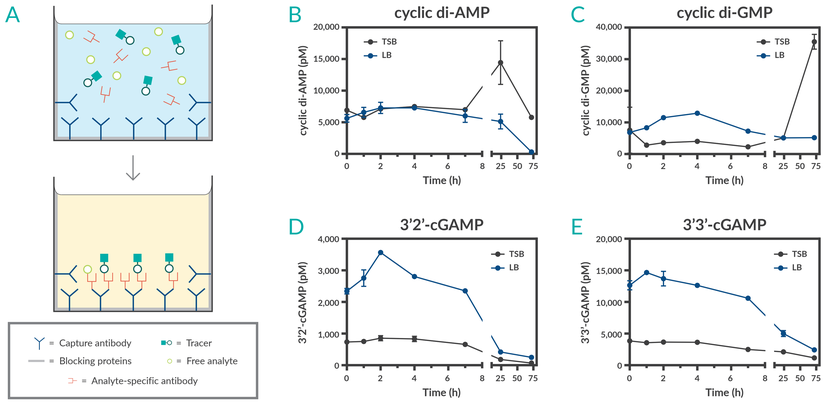

Figure 3– Kinetics of CDN production in *P. mirabilis*.
(A) Competitive ELISA schematic. **(B-E)** Overnight *P. mirabilis* cultures were normalized to an OD of 1.0, inoculated into the assay plate at final OD of 0.05, and incubated statically at 37°C for 72 hours. At the indicated timepoints, culture media were collected and CDN levels were quantified by competitive ELISA: **(B)** cyclic di-AMP ELISA Kit (Item No. 501960); **(C)** cyclic di-GMP ELISA kit (Item No. 501780); **(D)** 3'2'-cGAMP ELISA kit (Item No. 502340); and **(E)** 3'3'-cGAMP ELISA kit (Item No. 502130). N=2

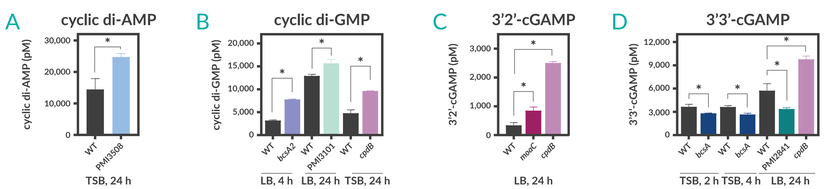

Figure 4 – CDN production significantly differed from WT in seven transposon mutant strains.
(A-D) Overnight cultures were normalized to OD 1.0, inoculated into the assay plate at final OD 0.05, and incubated statically at 37°C for 72 hours. *= p <0.05; N=2

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Swarming Motility

Overnight *P. mirabilis* cultures were normalized to OD 1.0 in PBS, 5 µl of which was spotted onto a swarm agar plate (per liter: 10 g tryptone, 10 g NaCl, 5 g yeast extract, 15 g agar). Once dry, the plates were incubated for 18 hours at 30°C before measurement of swarm ring diameters.

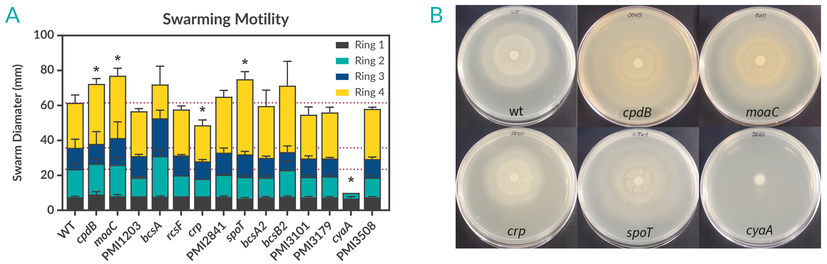

Figure 5 – Mutation of *crp* and *cyaA* inhibited swarming motility.
(A) Swarm diameters of *P. mirabilis* transposon mutants. **(B)** Representative images. *= p <0.05; N=3

HEK293T/17 Cell Adherence

To determine if mutations affect *P. mirabilis'* ability to adhere to human kidney cells, overnight cultures were first normalized to OD 1.0 in PBS and seeded at MOI of 200 onto confluent HEK293T/17 cells. Mammalian and bacterial cells were incubated together in DMEM for 2 hours at 37°C with 5% CO_2. Following incubation, mammalian cells were gently washed with PBS, lysed with 0.5% Triton X-100, and the number of bacterial colony forming units (CFU) was determined. CFU values were normalized to total protein levels and displayed relative to WT.

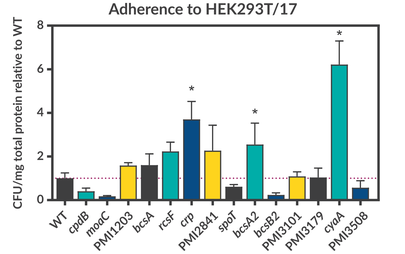

Figure 6 – Mutation of *crp*, *bcsA2*, and *cyaA* increased adherence of *P. mirabilis* to human kidney cells. N=3

CONCLUSIONS

- Mutation in the *moaC* gene, which encodes a putative phosphodiesterase, had increased biofilm formation, increased 3'2' cGAMP production, and a defect in swarming motility.
- Highly conserved transcriptional regulator *crp* and adenylate cyclase *cyaA* had a reciprocal defect in swarming motility/increased cellular attachment, however, *crp* was biofilm deficient.
- Three members of the bcs operon (*bcsA2*, *bcsB2*, PMI3101) annotated as important for cellulose biosynthesis had biofilm defects in multiple media despite increased cyclic di-GMP production.

References

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Acknowledgements

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