# Constructing a transposon library in clinical multidrug-resistant *P. aeruginosa*

**Yuanbiao Mou**
[1]Major in biological Sciences
[2]Research Mentors: Dr. Aixin Yan (Supervisor) and Dr. Zeling Xu, both from the School of Biological Sciences

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**Poster No.: F14**
**Name: Mou Yuanbiao**
**University No.: 3035437721**
**Student's Major: Biological Sciences**

## Introduction

*Pseudomonas aeruginosa* is a ubiquitous bacterium that causes severe infections in immune-compromised patients. A previous study isolated a multidrug-resistant (MDR) *P. aeruginosa* strain PA154197 from a local blood stream infection. The strain showed elevated production of the key virulence factor pyocyanin compared to the *P. aeruginosa* reference strain PAO1, alongside a unique quorum sensing (QS) pathoadaptation mechanism. To better understand how pyocyanin biosynthesis is regulated in PA154197, we constructed a lacZ fusion strain and performed insertional mutagenesis to search for regulators of pyocyanin synthesis.

## Key Findings

- β-galactosidase activity from the P*phz-lacZ* fusion gene predicts pyocyanin production
- Transposon (Tn) insertion in the *fimS* gene locus dramatically reduces pyocyanin production
- Deletion of *fimS* in wild-type *P. aeruginosa* did not lead to pyocyanin production inadequacy

## Figures

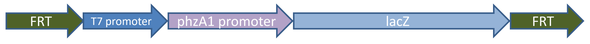


Figure 1. Schematic of the Pphz-lacZ fusion, flanked by Flp recombinase targets (FRTs) which facilitate Flp-mediated excision of the plasmid backbone (not shown) following chromosomal integration

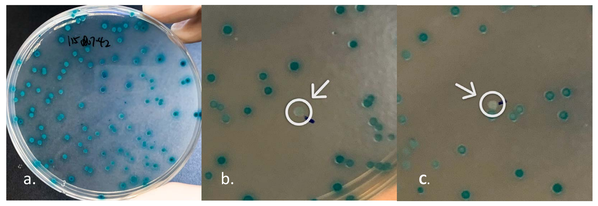


Figure 2. a. Phenotypic plates with TnM-PA154197 colonies post-incubation
b. c. light-colored colonies were selected for additional screening (encircled)

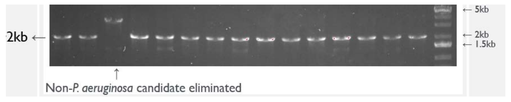


Figure 3. PCR amplification of a *Pseudomonas* exclusive gene cluster RhlI eliminated false positives, including stray donor *E. coli* SM10 colonies.

## Fusion Strain Construction

In *P. aeruginosa*, the phenazine biosynthetic operon *phzA1* primarily controls pyocyanin synthesis and is tightly regulated by the complex signaling network of the bacterium. We constructed a *lacZ* transcriptional fusion with the *phzA1* promoter and integrated it into the PA154197 chromosome to obtain a reporter strain in which β-galactosidase activity corresponds with pyocyanin production.

## Transposon Mutagenesis and Screening

We mutagenized our fusion strain with whole-genome transposon insertions using a Himar1-based transposon vector to generate a transposon library of PA154197. We selected for reduced-β-galactosidase activity mutants from a pool of 19,198 mutant colonies, based on their coloration when grown in indicator medium. 154 candidates were selected from this screening process, and their pyocyanin production levels were assayed to identify deficiency strains. Sequencing of the transposon junction revealed that TnM (Mariner transposon) insertion in the *fimS* locus significantly reduced pyocyanin production, showing a sevenfold decrease compared to the wild-type.

## Targeted Deletion

To characterize the function of *fimS*, we constructed a mini-CRISPR cassette containing a spacer sequence derived from *fimS*, sandwiched between repeats. Cloning this cassette into the platform plasmid AY5211 yielded a targeting plasmid capable of inducing chromosomal cleavage via the endogenous type I-F CRISPR-Cas system in *P. aeruginosa*. An editing plasmid was obtained by cloning two repair donor fragments into the targeting plasmid end-to-end. This plasmid was transformed into wild-type *P. aeruginosa* by conjugation. Chromosomal cleavage followed by homologous recombination yielded PA154197 *ΔfimS* deletion strain.

## Discussion

Interestingly, PA154197 *ΔfimS* displayed near-normal pyocyanin production levels. Previous studies in PAO1 had found that FimS acts as a cognate sensor for the downstream adjacent transcriptional regulator AlgR. AlgR may negatively modulate pyocyanin production by upregulating expression of czcR, whose product directly binds to the *phzA1* promoter. Removing the *algR* cognate sensor *fimS* could deregulate *czcR*, increasing pyocyanin production.
In such circumstances, the TnM insertion might positively regulate *algR* through polar effects to suppress pyocyanin production. Inside the transposon cassette a pTac and aaC1 promoter potentially activate transcription of surrounding genes. Activation of *algR* could suppress pyocyanin production through the CzcR-CzcS system. Further study is required to understand the role of the *fimS-algR* locus in pyocyanin virulence, beginning with deletion of the global regulator *algR* in PA154197.

## Figures


Figure 4. chart. Pyocyanin production levels at 48h into incubation
a. b. Visual comparison of the green pigment dispersed in culture

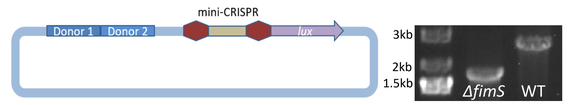


Figure 5. *left.* The *fimS* editing plasmid, with repair donors highlighted in blue
*right.* PCR confirmation of *fimS* deletion with primers flanking the 1kb gene

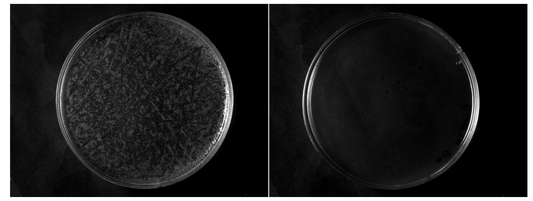

Figure 6. *left.* AY5211 transformants were recovered (control)
*right.* No colonies were recovered in targeting plasmid transformants

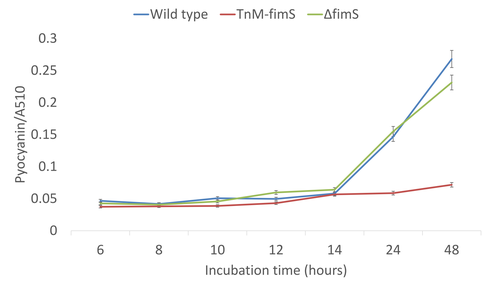


Figure 7. Pyocyanin production in wildtype, TnM-*fimS*, and *ΔfimS* variants of PA154197

## Key References

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