Virginia Commonwealth University
VCU Scholars Compass

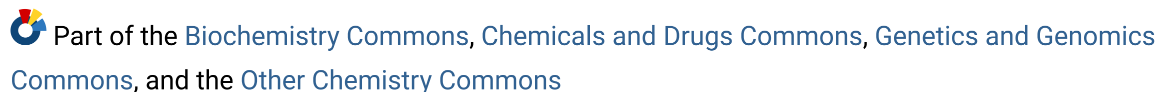
Graduate Research Posters Graduate School

2021

Transcriptional Repressor Protein based Macrolide Biosensor Development with Improved Sensitivity

Jayani A. Christopher

Follow this and additional works at: https://scholarscompass.vcu.edu/gradposters



Part of the Biochemistry Commons, Chemicals and Drugs Commons, Genetics and Genomics Commons, and the Other Chemistry Commons

Downloaded from

Christopher, Jayani A., "Transcriptional Repressor Protein based Macrolide Biosensor Development with Improved Sensitivity" (2021). *Graduate Research Posters.* Poster 118.
https://scholarscompass.vcu.edu/gradposters/118

This Poster is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Graduate Research Posters by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.

VCU Graduate School

Transcriptional Repressor Protein based macrolide biosensor development with improved sensitivity

Jayani A. Christopher, Ashton Cropp*

Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284

Introduction

Macrolides, a class of antibiotics are biosynthesized via "giant assembly line polyketide synthases(PKS) which could be modified by Combinatorial biosynthetic methods. However, it is challenging due to the size and complexity of PKSs. To overcome this, directed evolution can be used where a large libraries of enzyme variants need to be screened. It is important to develop high throughput screening methods to identify the enzymatic variants which can produce novel macrolides. MphR is a macrolide sensing transcriptional repressor protein which regulates a gene cassette where GFP is expressed upon binding of the macrolide ligand to MphR. This research is an insight of improving the sensitivity of MphR biosensor. Developing this system can address the need of novel macrolide antibiotic derivatives in the case of drug discovery.

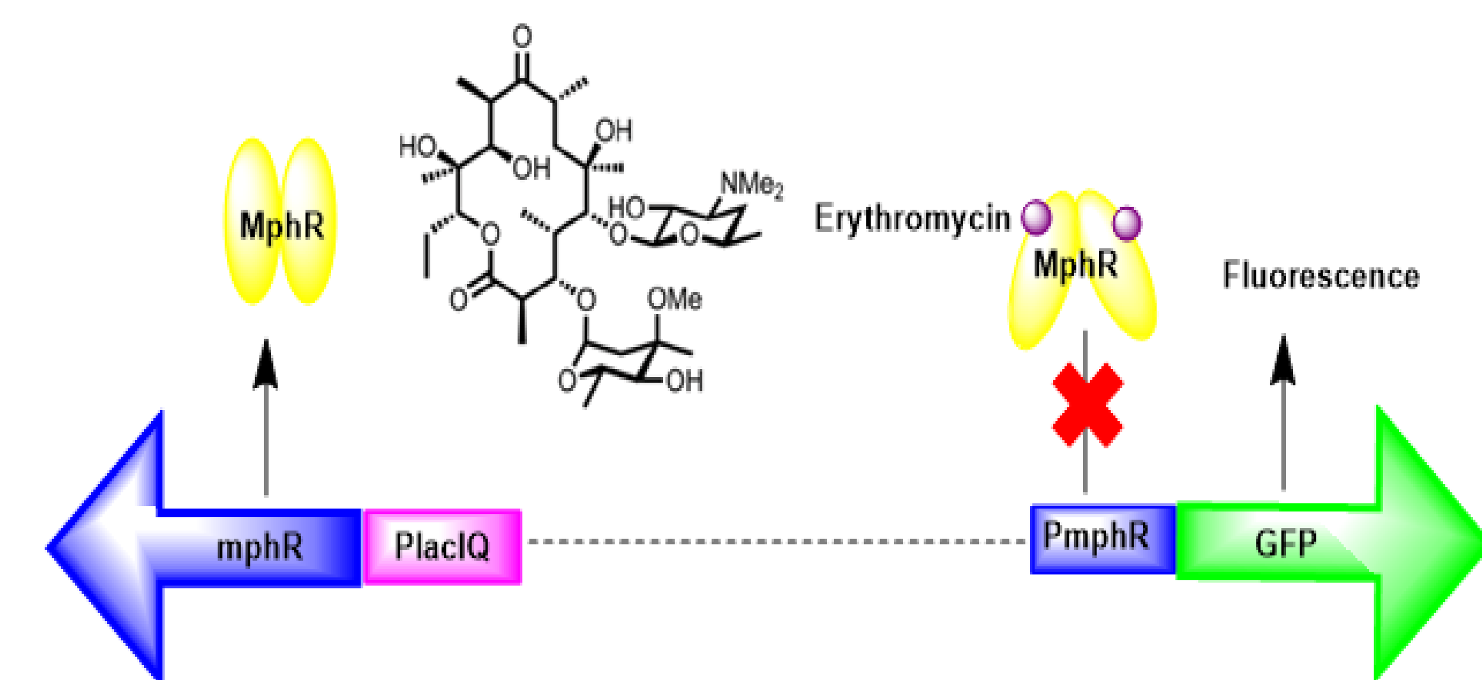


Figure 1. Schematic illustrating the role of MphR protein regulating the gene cassette containing GFP

Experimental Design and Results

Gene knockouts is a method of disrupting and inactivating chromosomal genes so the respective proteins are no longer expressed. This phenomenon has been used to obtain high sensitivity of the MphR biosensor.

TolC is a member in AcrAB-TolC multidrug efflux pump which can exclude the drugs from the cell. Knocking out the genes expressing TolC from the bacterial chromosome can retain more antibiotic in the cell to be detected by the biosensor.

BamB is a lipoprotein in the β barrel assembly machinery (BAM) which is involved in folding and insertion of outer membrane β barrel proteins. Knocking out bamB gene can reduce the membrane integrity leading to increased permeability for macrolides.

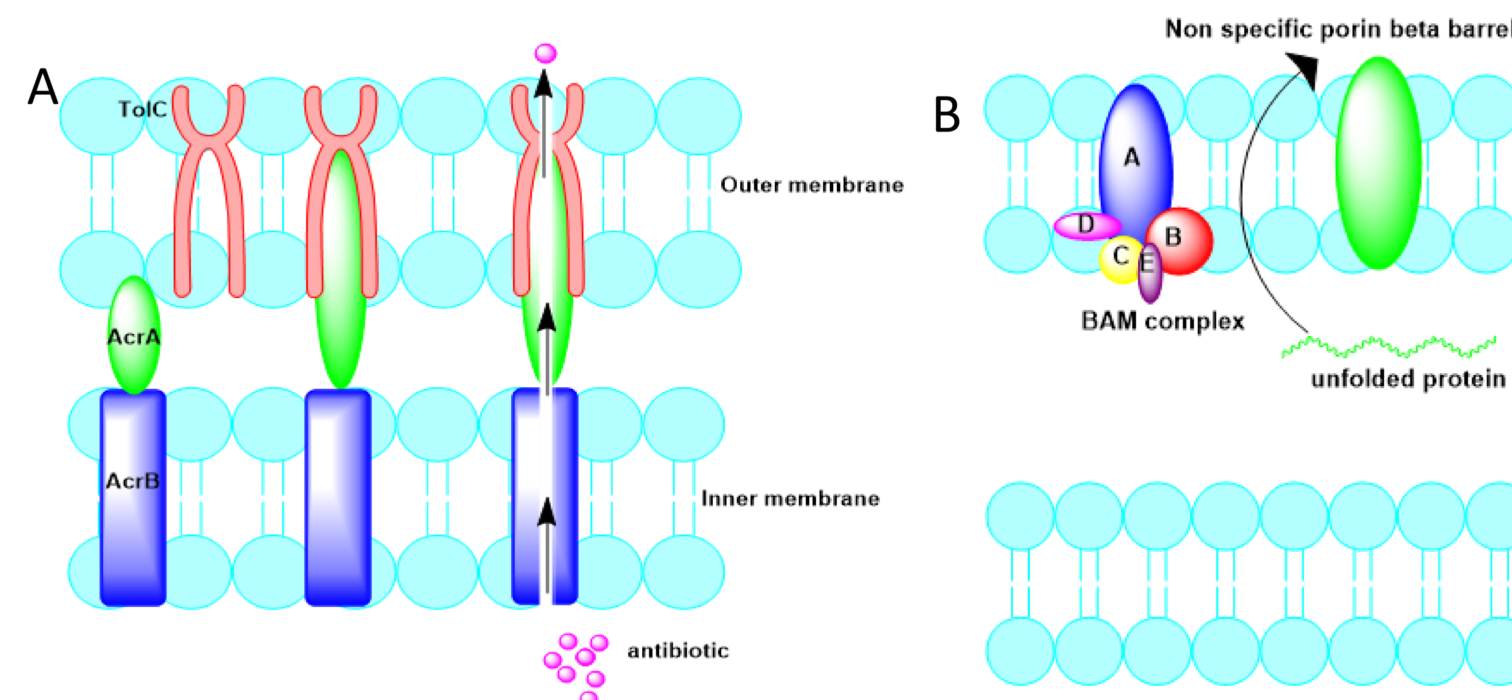


Figure 2 . Schematic illustrating the mechanism of A)AcrAB-tolC efflux pump B) Bam complex

GFP bioassays

- pMLGFP (MphR) +pJZ12 (MphA)/pJZ13 (MphA+mrX)/pACYC186 (No MphA or mrX)
- smRBS1A1 (mutated pMLGFP) +pJZ12/pJZ13/pACYC186 are transformed in tolC and bamBtolC knockout strains.

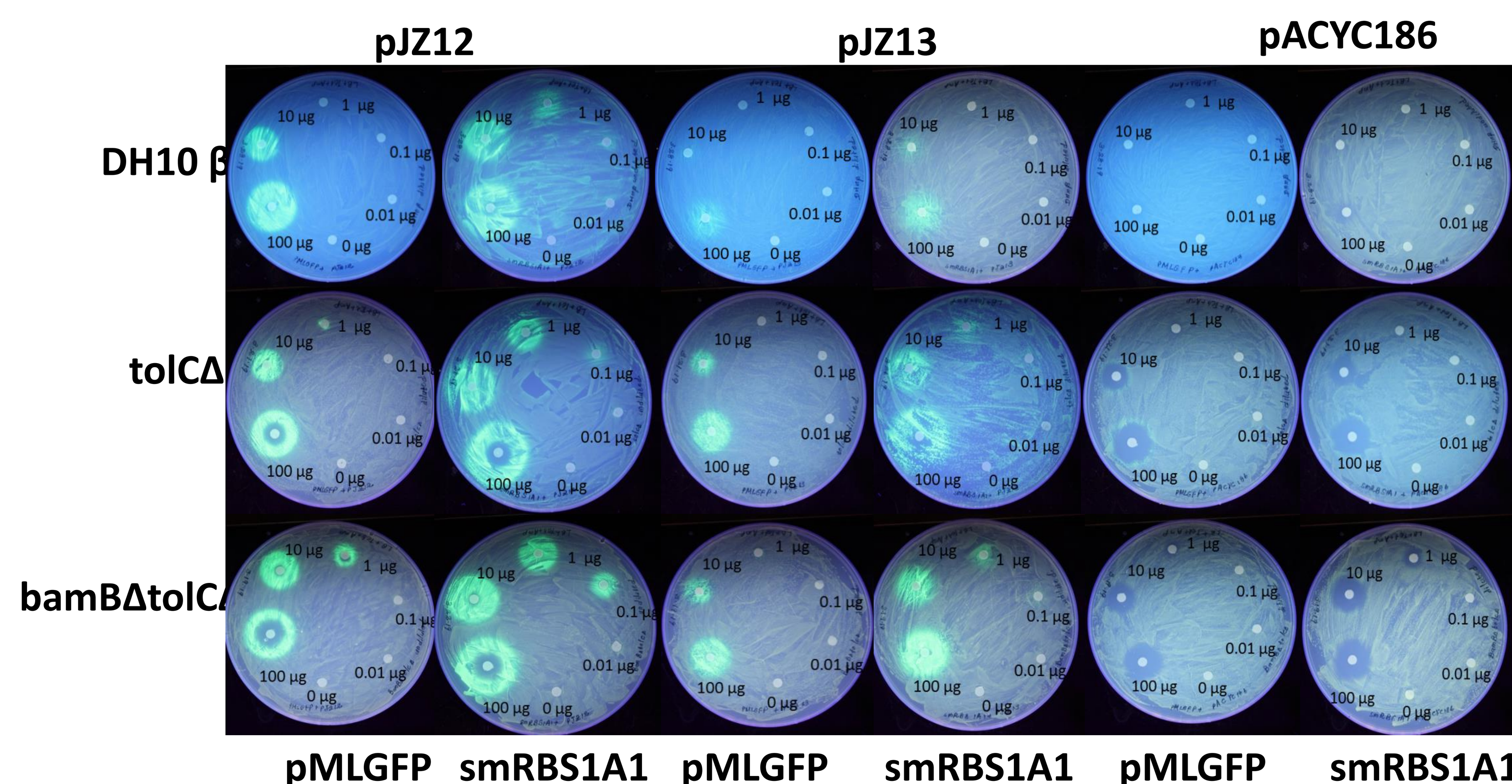


Figure 3. Agar diffusion assay plates with 2 plasmid systems in wild type, single and double gene knockout *Ecoli*

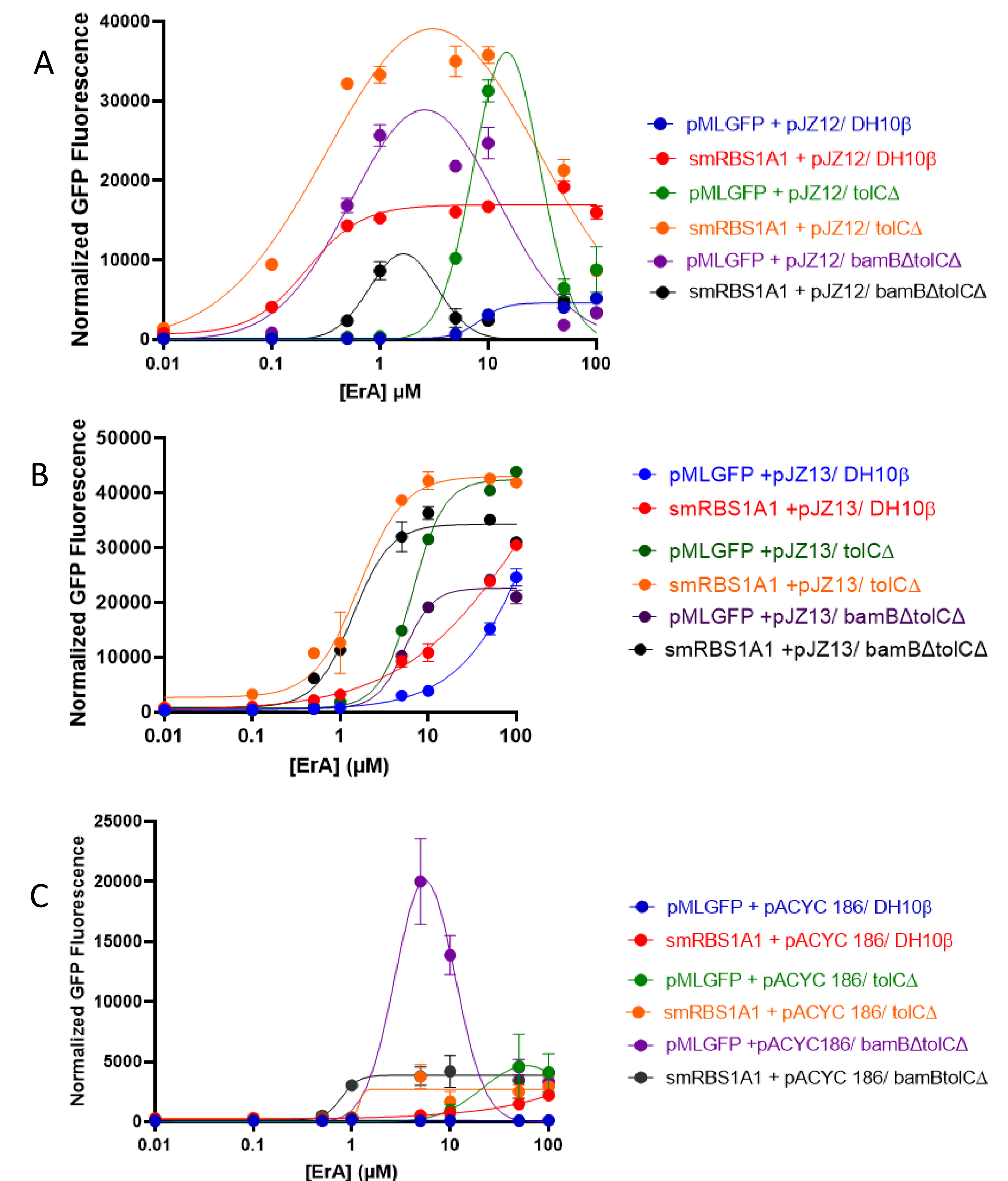


Figure 4. . Dose response curves of A) pJZ12 strains B) pJZ13 strains) C)pACYC186 strains

Conclusions

- Gene knockouts improves the sensitivity of the biosensor and erythromycin can be detected down to 0.1 μg with smRBS1A1 + pJZ12/ bamBΔtolCΔ strain.
- Dynamic range is reduced with the double knockout strains due to cell death at high concentrations of erythromycin.

References

1. *Kasey et.al. Development of Transcription Factor-Based Designer Macrolide Biosensors for Metabolic Engineering and Synthetic Biology.* ***2018**.*.

2. *Wang et.al. An Allosteric Transport Mechanism for the AcrAB-TolC Multidrug Efflux Pump.* ***2017**, 1–19.*

3. *Jiang et.al. From Evolution to Pathogenesis : The Link Between β -Barrel Assembly Machineries in the Outer Membrane of Mitochondria and Gram-Negative Bacteria.* ***2012**, 8038–8050.*