Efflux Pumps Contributing to Antibiotic-Resistance are Conserved Across Species of *Pseudomonas*

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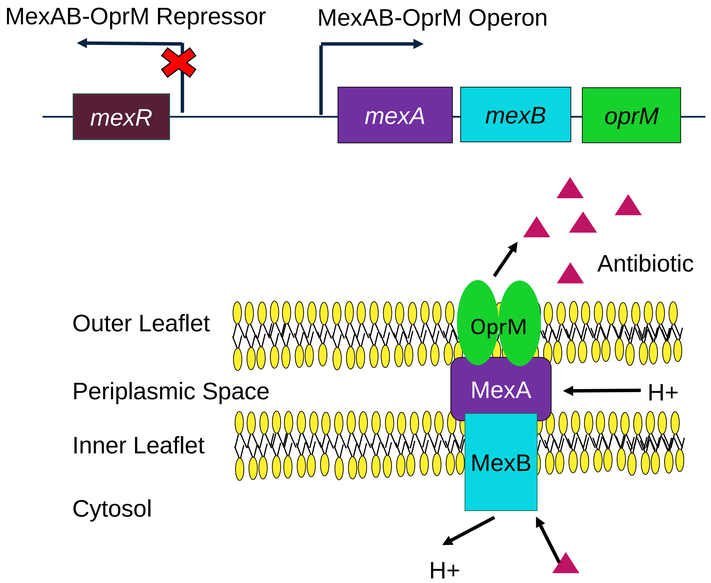
Introduction

Pseudomonas aeruginosa is an antibiotic resistant and opportunistic pathogen that commonly causes infections in cystic fibrosis patients. *P. aeruginosa* contains an intermembrane efflux pump encoded for by the *mexAB-oprM* operon that is responsible for expulsion of antibiotics and other toxins. Resistance is often caused by mutations in the MexAB-OprM repressor which creates increased expression of the *mexAB-oprM* operon.



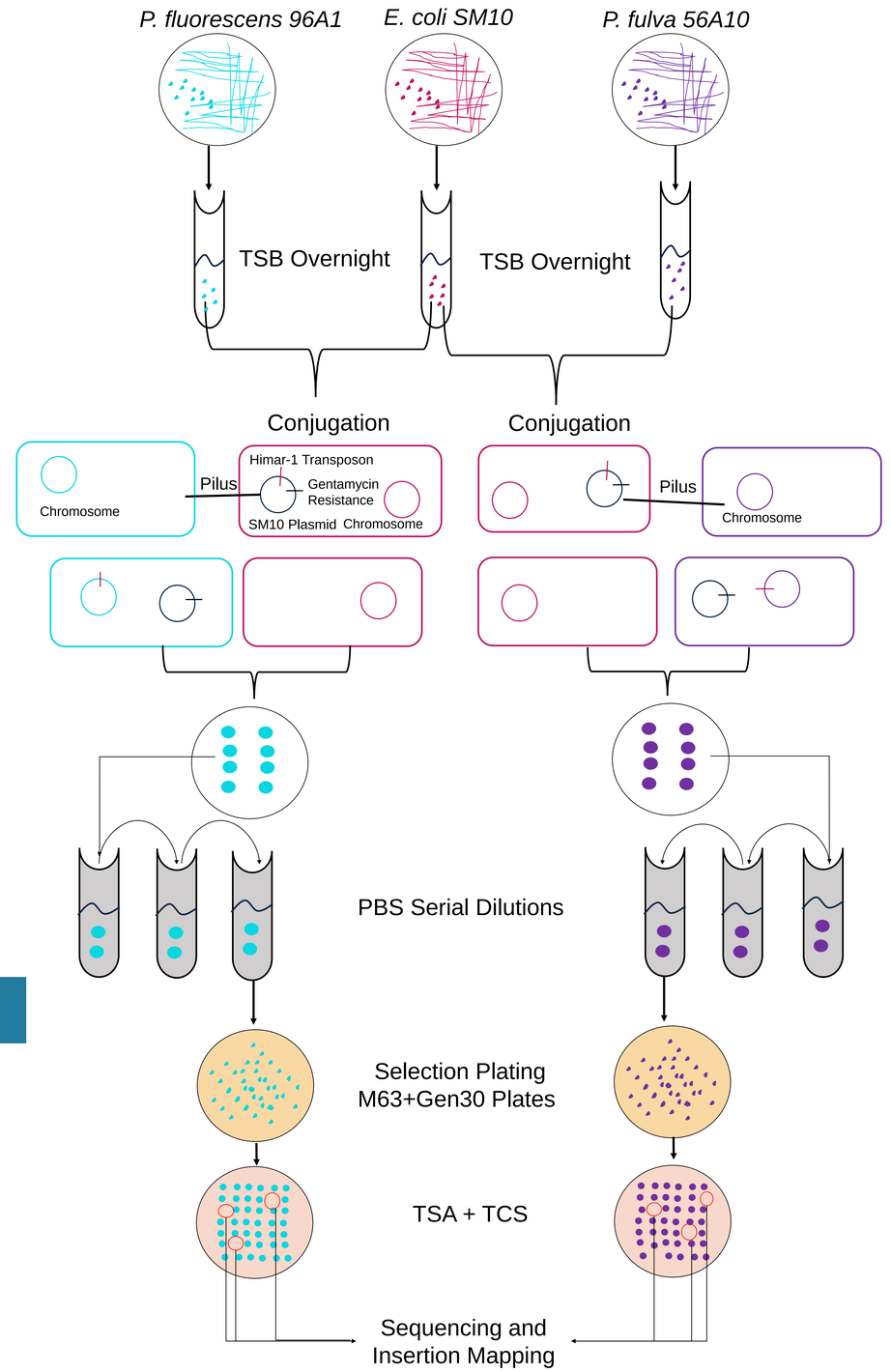
Goal and Hypothesis

Goal ➡ To asses the genetic factors involved in Triclosan (TCS) resistance in *P. fluorescens* and *P. fulva*

Hypothesis ➡ Resistance in these strains is determined by their MexAB-OprM homolog and interruptions or mutations in the operon will result in a loss of resistance

Methods

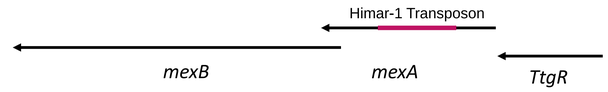
Transposon Mutagenesis



Results and Analysis

P. fulva Screening Statistics

Set	Colonies screened	Potential hits	TCS-Sensitive colonies
1	461	5	0
2	375	0	0
3	468	0	0
4	187	1	p2s4-A12
5	900	0	0
6	900	0	0
7	810	2	s7-8-B2, s7-8-H5
Total:	4101	8	3



P. fulva Minimal Inhibitory Concentration (MIC)

TCS Concentration	128 mg/L	64 mg/L	32 mg/L	16 mg/L	8 mg/L	4 mg/L	2 mg/L	1 mg/L	0.5 mg/L	.25 mg/L	.125 mg/L	.0625 mg/L
56A10 WT	0.556	0.564	0.623	1.033	1.39	1.432	1.434	1.38	1.351	1.345	1.338	0
56A10 WT	0.507	0.409	0.339	0.592	1.258	1.433	1.431	1.348	1.329	1.319	1.323	0
56A10 WT	0.546	0.499	0.346	0.701	1.247	1.421	1.392	1.36	1.319	1.277	1.314	0.001
56A10-p2s4-A12	0.006	0.006	0.004	0.11	0.012	0.542	1.204	1.207	1.22	1.221	1.23	0
56A10-p2s4-A12	0.006	0.006	0.005	0.002	0.006	0.455	1.184	1.248	1.252	1.25	1.259	0
56A10-p2s4-A12	0.005	0.006	0.005	0.002	0.08	0.642	1.226	1.312	1.281	1.259	1.26	0

Low MIC: Low Resistance

High MIC: High Resistance

Wild Type MIC: 128.00 mg/L
Tn Mutant MIC: 8 mg/L

Conclusions

Knocking out the *mexA* gene through transposon mutagenesis successfully decreased triclosan resistance in *P. fulva,* indicating that the MexAB oprM efflux pump and the mechanism of resistance in *P. aeruginosa* is highly conserved between these two strains.

References

Oliver, Antonio, et al. “High Frequency of Hypermutable Pseudomonas Aeruginosa in Cystic Fibrosis Lung Infection.” *Science*, American Association for the Advancement of Science, 19 May 2000, science.sciencemag.org/content/288/5469/1251.

Lambert, P A. “Mechanisms of Antibiotic Resistance in Pseudomonas Aeruginosa.” *National Center for Biotechnology Information*, Journal Of The Royal Society Of Medicine, 2002, www.ncbi.nlm.nih.gov/pmc/articles/PMC1308633/pdf/12216271.pdf.

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