Identification of a Novel Gene Involved in *Pseudomonas aeruginosa* Biofilm-Specific Resistance to Antibiotics

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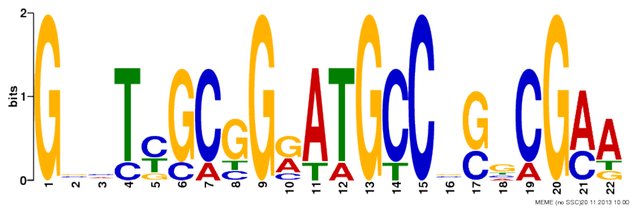
Introduction

Bacteria growing in biofilms are the leading cause of hospital-acquired implant-based infections and the basis of many persistent diseases, such as otitis media, periodontitis and lung infections in cystic fibrosis patients. The persistence of these infections is due to the recalcitrance of biofilms to the immune system and antimicrobial agents. Biofilm cells are more resistant to antibiotics (10 to 100 times) than planktonic (free-floating) bacteria. This increase in antibiotic resistance is due to multiple intrinsic mechanisms of resistance that act together to provide an increased overall level of resistance. Some mechanisms that contribute to the overall antibiotic resistance in a biofilm are mediated by the extracellular matrix, quorum sensing signalling, and stationary phase stress resistance induced by oxygen and/or nutrient deprivation.

We hypothesize that there are additional mechanisms of resistance directed by genes expressed in a biofilm-specific manner.

Background

We have been analyzing 6 *Pseudomonas aeruginosa* biofilm-specific antibiotic resistance genetic loci. Mutation of any one genetic locus results in a 2-4 fold reduction in biofilm antibiotic resistance. These genes, *ndvB,* PA1875-77, *tssC1*, PA0756-757, PA2070 and PA5033, are all more highly expressed in biofilms compared to planktonic cultures, suggesting that there is a common mechanism of regulation. *In silico* analysis has revealed a **22-base pair consensus sequence** in the promoters of these 6 loci.



Subsequent BLAST analysis has identified 11 additional loci whose predicted promoters also contain this consensus sequence. These 11 uncharacterized genes likely represent new biofilm-specific antibiotic resistance genes. We have initiated our analysis of these genes by studying a transposon-insertion mutant of PA1993. Mutation of PA1993 did not affect biofilm formation, growth under standard conditions or planktonic antibiotic resistance. However, PA1993tn displayed increased sensitivity to ciprofloxacin, specifically when the mutant strain was growing in a biofilm.

Materials and Methods

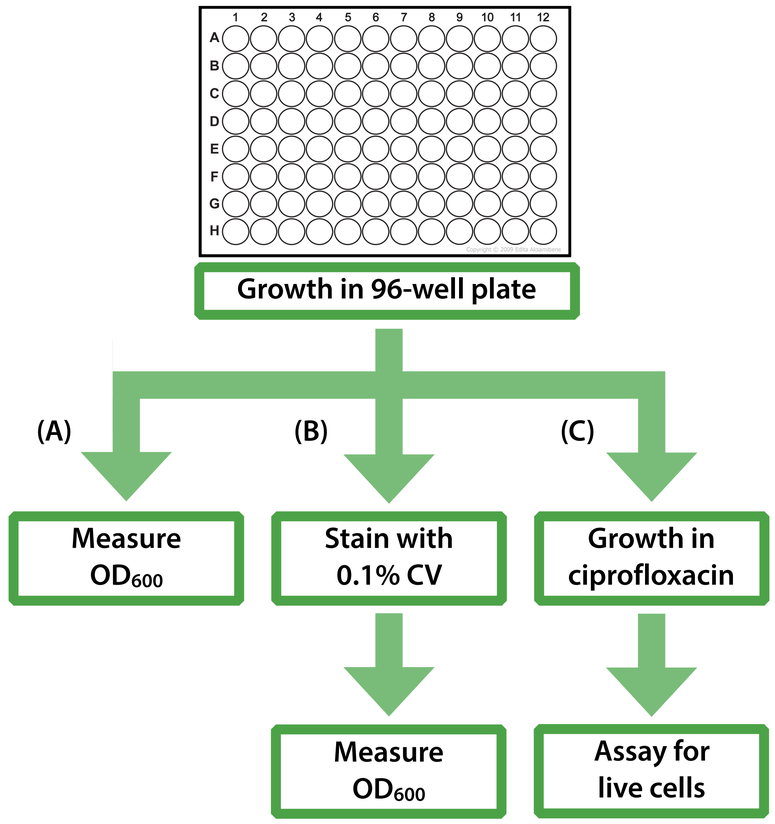


Figure 1. Methodology of techniques used in project. **(A) Growth assay:** tested for effects on growth in LB and M63 due to transposon insertion. **(B) Biofilm formation assay:** tested for effects on the ability to form biofilms due to transposon insertion. **(C) Minimal bactericidal concentration assays:** tested for antibiotic sensitivity in planktonic cultures (MBC-P) and biofilms (MBC-B).

Results

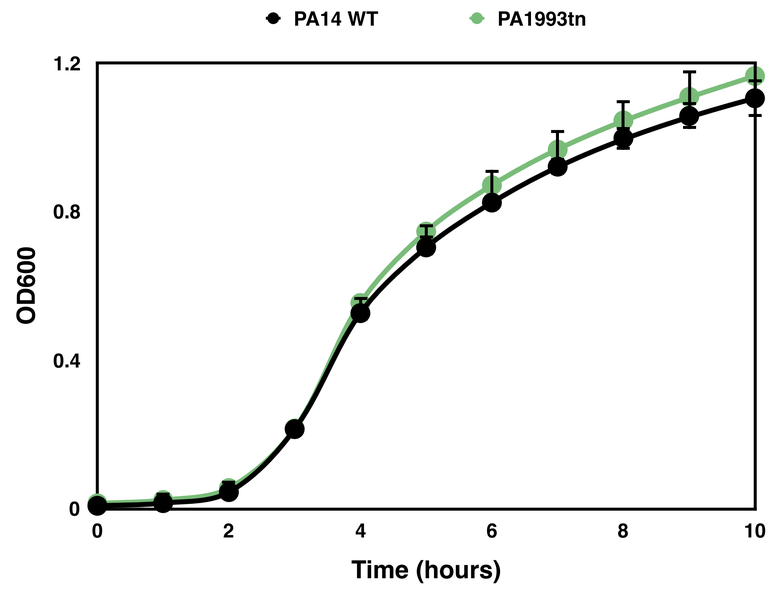


Figure 2. Growth of strains in LB. Representative growth curve is shown. Error bars denote SD per replicates in plate (n = 6).

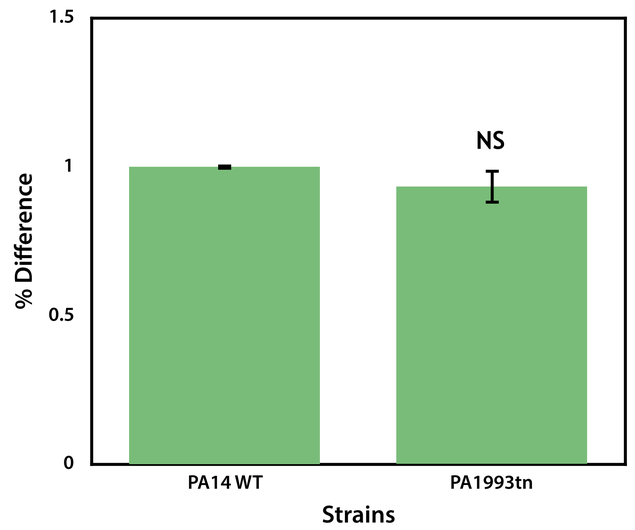


Figure 3. Biofilm formation assay. Normalized to percent difference of OD_{600} compared to WT. NS represents no significant difference. Error bars denote SD (n = 3).

Strain	MBC-P (µg/mL)	MBC-B (µg/mL)
PA14 WT	2	10
PA1993tn	2	5

Table 1. Minimal bactericidal concentrations of planktonic (MBC-P) and biofilm (MBC-B) cells with the antibiotic ciprofloxacin.

Conclusions

Our data support the hypothesis that PA1993 is involved in biofilm-specific antibiotic resistance. The PA1993tn mutant displays no defect in growth, biofilm formation or planktonic antibiotic resistance. However, this mutant is more sensitive to ciprofloxacin in a biofilm-specific antibiotic resistance assay.

Future work will consist of comparing PA1993 gene expression in planktonic and biofilm populations, and confirming observations in a ΔPA1993 mutant.

Acknowledgements

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