Effects of Daptomycin in Streptococcus pneumoniae: Verifying phenotypes within single gene knockouts

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2014

Effects of Daptomycin in *Streptococcus pneumoniae*: Verifying phenotypes within single gene knockouts

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Introduction

- **The Bacteria:** *Streptococcus pneumoniae* is a Gram-positive bacterial pathogen, found commonly in the nasopharynx
- **Large Genetic Diversity:** *S. pneumoniae* consists of 90 serotypes, with each strain containing more than 220 unique genes.
- **High-throughput genome-wide methodology:** Tn-seq is a genome sequencing methodology which utilizes a fully saturated transposon library. Previously produced data by this method informs the selection of significant genes in an antibiotic stress environment.
- **The Antibiotic:** Daptomycin is "novel antibiotic" in the lipopeptide class. The antibiotic lacks a specific target, but through accumulation causes membrane distortion which leads to release of potassium and subsequently cell death.
- **Threat to Health:** Upon colonization, *S. pneumoniae* can cause middle ear infections (otis media), meningitis, pneumonia, and bactremia (infection of the blood)
- Pneumonia alone is implicated in 13% of post-neonatal and 2% of neonatal deaths[2] globally and 19% of under five mortalities[3] making *S. pneumoniae* a research target for reaching Millennium Development Goal 4A to reduce by two thirds, between1990 and 2015, the under-five mortality rate.

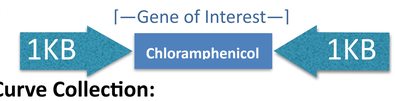
Methods

Determining Genes of Interest:

- Two strains of *S. pneumoniae* were used: Taiwan 19F and TIGR4
- Tn-Seq data was analyzed for significant defect or advantage in Daptomycin
- Fourteen genes of interest were selected (indicated in Figures 1 and 2)

Construction of Gene Knockouts:

- Using PCR amplification and annealing a 3KB construct consisting of two 1KB regions flanking the gene of interest and a chloramphenicol drug marker were transformed into wild type strains of TIGR4 (2394) and Taiwan 19F (see diagram below)



Growth Curve Collection:

- Mutant and wild type strains were grown in Semi-Defined Minimal Media in the presence of Daptomycinover a 16-hour period using optical density, collected every 30 minutes, as a determinate of growth
- Mutant phenotype was verified through comparison of these data with Tn-Seq analysis

Acknowledgements

Primarily, much gratitude is owed to Tim van Opijnen and Sandra Dendrick who have acted as mentors and guides in this research. Many thanks to the van Opijnen lab team, in particular, Karen Zhou, Derek Thibault, and Paul Jensen for their patience, training, and support.

2 http://www.who.int/topics/millennium_development_goals/child_mortality/en/
3 http://www.unicef.org/mdg/mortalitymultimedia/

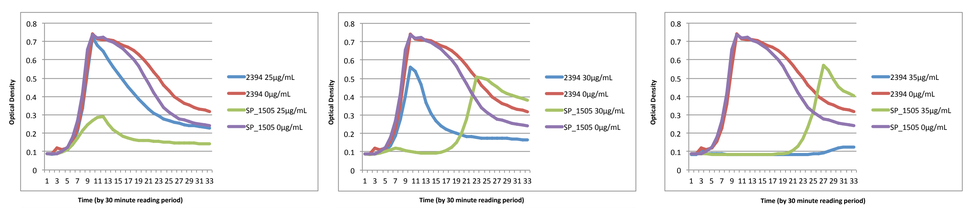
Table 1- Tn-seq fitness analysis in selected knockout mutants of interest

TIGR4	Fitness in Glucose	Fitness in Daptomycin	*Function*	*Taiwan 19F*	Fitness in Glucose	Fitness in Daptomycin	*Function*
ΔSP_1505	*0.950*	*0.169*	*Membrane Protein*	*ΔSPT_1441*	*0.984*	*0.256*	*Transport*
ΔSP_1506	*0.682*	*0.855*	*Carbohydrate Metabolism*	*ΔSPT_1442*	*0.839*	*0.939*	*Cell Division*

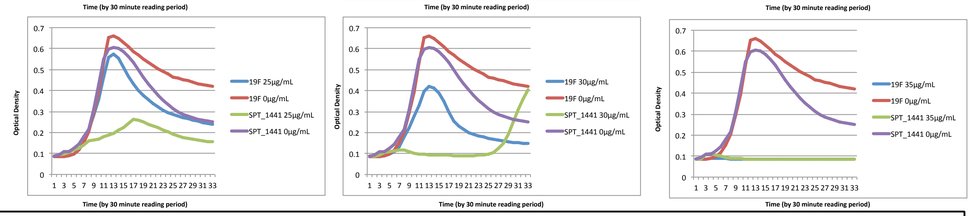
Discussion

- As is evident by Figure 1 and Figure 2, the fourteen genes of interest discussed in this study are representative of the diverse function gene groups.
- Knowledge of the mechanism of Daptomycin induced cell death indicates that genes involved in membrane construction or transport are likely to be important in bacterial cell resistance or tolerance in the presence of the antibiotic
- In contrast to the defect phenotype verified above, knocking out some genes led to advantageous fitness.
- There is not a singular correlation between functional group and advantageous or disadvantageous fitness, further attributing to the complexity of the mechanisms of resistance

Graph Set A

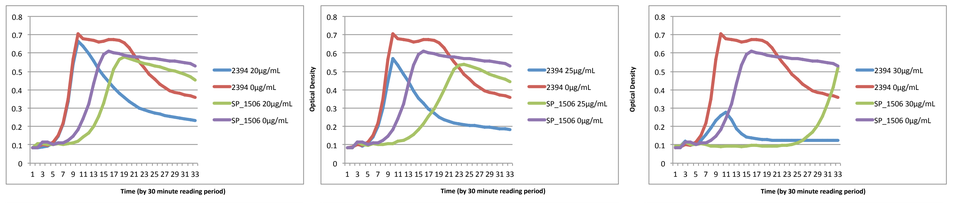


Graph Set B

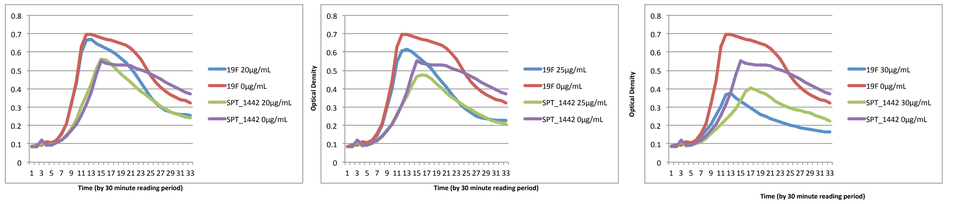


The hypothesized significance of membrane related genes is exhibited by homologous gene knockouts SP_1505 (Graph Set A) and SPT_1441 (Graph Set B) for which a drastic defect was confirmed phenotypically

Graph Set C



Graph Set D



Advantageous fitness is exhibited by SPT_1442 mutants (Graph Set D) which have improved survival in Daptomycin relative to their wild type survival in glucose. Its function is cell division. The homologous gene in TIGR4 whose functionality is carbohydrate metabolism, SP_1506 (Graph Set C), exhibits a more severe relative defecit in glucose than Daptomycin.

Figure 1

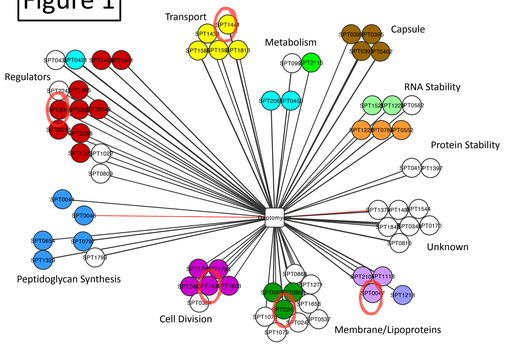
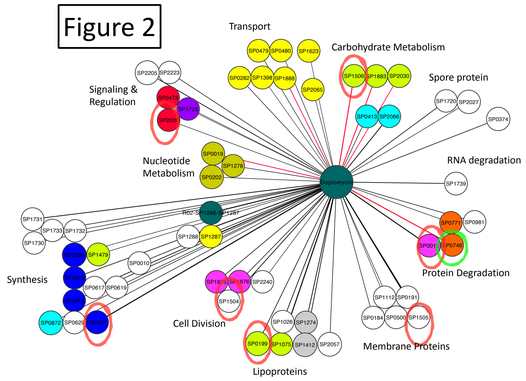


Figure 1 and Figure 2 depict Taiwain 19F (19F) and TIGR4 (2394), respectively, genome maps of genes of interest, organized by functionality ("function groups"). Encircled genes are those for which mutants were constructed and are encompassed in this study.

Genome Maps credit to Tim van Opijnen, 2013

Figure 2



Future Directions

- Our current focus on is on SP_0746/SPT_0761, a newly transformed knock out for which the phenotype is still being confirmed
- This gene codes for ClpP protease and Tn-Seq data indicates an advantageous phenotype which have made this gene of significant interest
- We are interested in gaining further understanding of the essential genetic pathway for survival *Streptococcus pneumoniae* in the presence of antibiotics
- Through high-throughput genome-wide methodology, in these two strains alone, over 200 genes of interest have been identified
- Using the outlined methodology we will continue to discover gene and antibiotic specific interactions and pathways
- Further research into the roles of these pathways is necessary to improve currently available antibiotics, and for the discovery of novel therapeutics.
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