Using transposon mutagenesis to discover new drug targets in *Burkholderia thailandensis*

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Background

Burkholderia pseudomallei is a human pathogen which causes the disease melioidosis and is resistant to a wide variety of antibiotics. Melioidosis is endemic to areas in southeast Asia and northern Australia and can manifest in a variety of ways including pneumonia, localized skin infections and septicemia[1]. Work completed in the Seyedsayamdost Lab has shown that in *B. thailandensis*, a non-pathogenic model for *B. pseudomallei*, low doses of the antibiotic trimethoprim (TMP) cause global secondary metabolism changes including an increase in the expression of the folate biosynthesis gene *folE2.* Subinhibitory doses of TMP in a knockout of the gene *folE2* cause a synthetic lethal phenotype[2].

Research Question

My project seeks to identify other genes that produce a synthetic lethal phenotype with TMP. Synthetic lethality occurs when an inactivation of a combination of genes that causes cell death but their independent inactivation does not. We extend our use of the term to include the use of compounds, which that the doses used, do not inhibit growth.

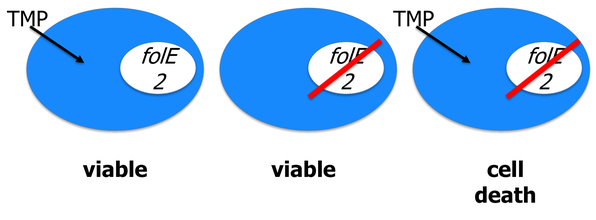

Figure 1: Example of synthetic lethality using TMP in *ΔfolE2* cells.

Methods and Materials

In order to identify new synthetic lethal interactions I constructed a T8, a modified Tn5, transposon library of *B. thailandensis* via conjugation with an *E.coli* super donor. The mutants are stored in 96 well plates at -80°C. To ensure 2-fold genome coverage, 12,000 mutants were collected. A schematic of the screen for synthetic lethality is shown in Figure 2.

Workflow

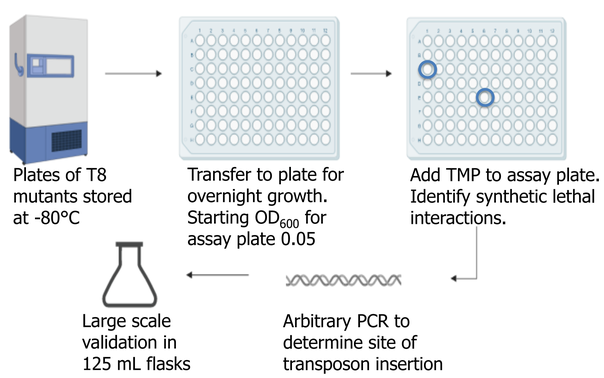

Figure 2: Schematic of TMP screen of T8 mutants.

Results

- ~6,000 mutants screened
- 116 initial hits

Table 1: Selection of initial hits

Locus	Product
BTH_I0357 (metH)	5-methyltetrahydrofolate--homocysteine methyltransferase
BTH_I0844 (cobQ)	cobyric acid synthase
BTH_I2394 (cobM)	precorrin-4 C11-methyltransferase
BTH_I2399 (cobH)	precorrin-8X methylmutase
BTH_I2407 (cobN)	cobaltochelatase subunit CobN
BTH_I0246 (flgH)	flagellar basal body L-ring protein
BTH_I0247 (flgI)	flagellar basal body P-ring protein
BTH_I0196 (fliJ)	flagellar FliJ protein
BTH_I0200 (fliF)	flagellar MS-ring protein
BTH_I3169 (flhA)	flagellar biosynthesis protein FlhA
BTH_I0199 (fliG)	flagellar motor switch protein G
BTH_I3184 (motB)	flagellar motor protein MotB
BTH_II1893 (vgrG-4b)	type VI secretion system protein TssI-4b
BTH_II1900 (tssC-4)	type VI secretion system protein TssC-4
BTH_II1901 (tssB-4)	type VI secretion system protein TssC-4
BTH_II1902 (tssA-4)	type VI secretion system protein TssC-4

Putative gene annotations from Burkholderia Genome Database

Discussion

As we continue to validate our current list of hits, some patterns are emerging and prove interesting.

- *cobH*, *cobN*, *cobQ* and *cobM* are all implicated in vitamin B12 synthesis which involves one-carbon metabolism. The mode of action of trimethoprim inhibits folate biosynthesis, a one-carbon metabolic pathway[4].
- *metH* encodes a vitamin B12 dependent enzyme responsible for part of the synthesis of methionine from homocysteine[5].
- There are multiple type VI secretion system (T6SS) proteins of the 4th subgroup that are also potential hits. While their function hasn't been elucidated, their relationship to other T6SS proteins suggest they have implications in interspecies communication. Other T6SS proteins have roles in virulence in both B. *pseudomallei* and B. *thailandensis*[3].

Future Directions

- Continue to screen and validate the rest of the transposon mutant library.
- Begin to follow up on hits with assays to determine the enzymes or proteins responsible for the synthetic lethal phenotype.
- Elucidate mechanisms of synthetic lethality with trimethoprim
- Find small molecule inhibitors of validated hits.

Acknowledgments

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References

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