Non-Essentiality of \textit{alr} and \textit{murI} Genes in Mycobacteria

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Non-Essentiality of *alr* and *murI* Genes in Mycobacteria

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Introduction

Amino acids are the building blocks of life. If DNA is the blueprint, amino acids are the lumber that proteins are built with. Proteins are built with left-handed, L-forms of amino acids. Bacteria have an essential cell wall component that happens to be an exception: peptidoglycan. Bacteria have enzymes called racemases that convert L-amino acid forms into right-handed, D-forms. Amino acids participate in many reactions with keto acids. For preliminary analysis, we have transformed the plasmids carrying Msmeg_5795 along with other control plasmids into wild type and mutant strains and evaluated growth in various media in the presence of different supplements. The mutant strain shown above, Tam29-12 has a mutant in both *alr* and *murI* genes. Strain 487 is transformed with an empty plasmid while strains 494, 495, and 535 are transformed with Msmeg_5795 (previously cloned in our lab) and Rv0812 (the object of this study) respectively.

Background

The Alanine and Glutamate Love-Square

L-Ala D-Ala
L-Glu D-Glu
Alr Glutamate dehydrogenase
Mur Enzyme for peptidoglycan synthesis

Glutamate and alanine are two vital amino acids for building peptidoglycan. In mycobacteria, the genes *alr*, *murI*, *lat*, and *dat* code for enzymes that can be used as shown above. We hypothesize that Lat and Dat, which have not been identified, provide redundant function in the absence of mutations in *alr* and *murI*. Bioinformatic analysis identified Msmeg_5795 (previously cloned in our lab) and Rv0812 (the object of this study) as the potential transaminases in *M. smegmatis* and *M. tuberculosis*, respectively.

Materials & Methods

Design PCR Primers
Retrieve gene sequence
Polymerase Chain Reaction
Transformation
Electroporate plasmid into *M. smegmatis*
Plate transformed cells on antibiotic media
Pick colonies
Restriction Digest
Digest plasmid
Ligate PCR product into plasmid
Digest PCR product containing gene Rv0812

Results & Discussion

Fig 1. Cloning of Rv0812 into plasmid pBUN421

Two common strains of Mycobacterium tuberculosis used in the laboratory are CDC1551 and H37Rv. CDC1551 is a more virulent strain than H37Rv. As the *alr* gene could be inactivated in CDC1551 but not in H37Rv, we hypothesize that the *dat* gene appears to be functional in CDC1551 (MT0833) but not in H37Rv (Rv0812). The purpose of this experiment was to extract the candidate *dat* genes from the respective organisms, insert them into the plasmid vector pBUN421, and transformed them into the model organism *M. smegmatis* for further study. Constructions are underway and will be confirmed by PCR and restriction analysis.

Fig 2. Growth analysis of multiple plasmids in *M. smegmatis*

For preliminary analysis, we have transformed the plasmids carrying Msmeg_5795 along with other control plasmids into *M. smegmatis* wild type and mutant strains and evaluated growth in various media in the presence of different supplements. The mutant strain shown above, Tam29-12 has a mutant in both *alr* and *murI* genes. Strain 487 is transformed with an empty plasmid while strains 494, 495, and 535 are transformed with Msmeg_5795 and Rv0812 respectively. Growth is observed on MADC when Msmeg_5795 is present. Compare to positive and negative controls, MADC with 5mM D-ala and LBT is inhibited by catabolite repression.

The observed results suggest that Msmeg_5795 may encode the *dat* gene but further experimentation and replication is necessary.

Future Directions

- Observe growth of additional *M. smegmatis* transformants utilizing plasmids constructed in Fig 1
- Transform other pathogenic mycobacterial species wild type and mutant strains with plasmids of interest
- Study growth and metabolism in the new transformants

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