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

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

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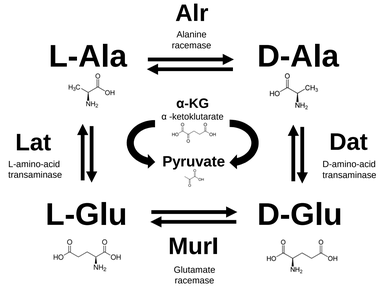
Introduction

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. Transaminases allow conversion between amino acids by transfer of an amino group.

Previous reports claimed there is no D-ala transaminase activity in mycobacteria and thus *alr* and *murI* genes encode essential functions. However, in studies performed by our lab, *alr* and *murI* mutants were able to grow on minimal or low-nitrogen content media. This suggests there is D-ala transaminase activity in mycobacteria and thus *alr* and *murI* genes encode essential functions.

Background

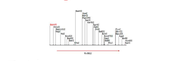
The Alanine and Glutamate Love-Square



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 M_{smeg_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

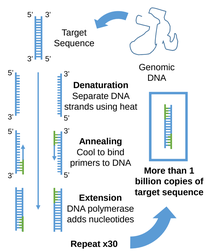
Polymerase Chain Reaction



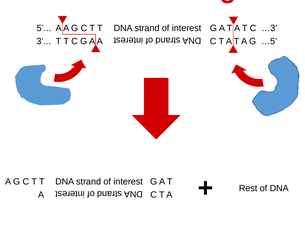
Retrieve gene sequence

Design PCR Primers

Run PCR on genomic DNA



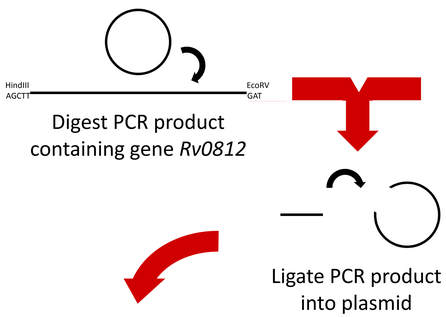
Restriction Digest

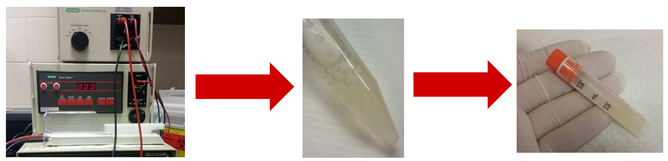


Results & Discussion

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 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.





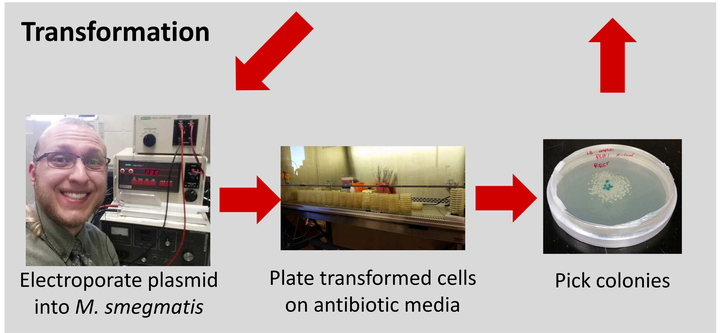
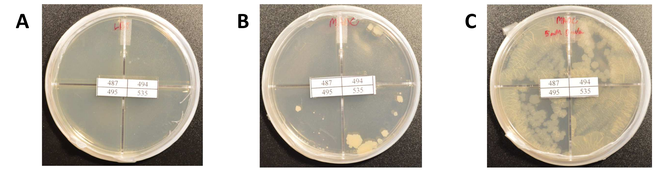


Fig 2. Growth analysis of multiple plasmids in *M. smegmatis*



A Twelfth-day growth of four transformants of *M. smegmatis* on LBT agar.
B Twelfth-day growth of four transformants of *M. smegmatis* on MADC agar.
C Twelfth-day growth of four transformants of *M. smegmatis* on MADC agar supplemented with 5mM D-ala.

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, Tam23-12 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 M_{smeg_5795} (*alr*). Growth is observed on MADC when M_{smeg_5795} is present. Compare to positive and negative controls, MADC with 5mM D-ala and LBT, respectively. Growth on LBT is inhibited by catabolite repression.

The observed results suggest that M_{smeg_5795} may encoded the *dat* gene but further experimentation and replication is necessary.

Future Directions

- Observe growth of *M. smegmatis* transformants on more diverse media conditions
- 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

Acknowledgments

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