

Spring 4-8-2016

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

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Hoff, Philion L.; Zinniel, Denise; and Barletta, Raúl G., "Non-Essentiality of *alr* and *murI* Genes in Mycobacteria" (2016). *UCARE Research Products*. 27.

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

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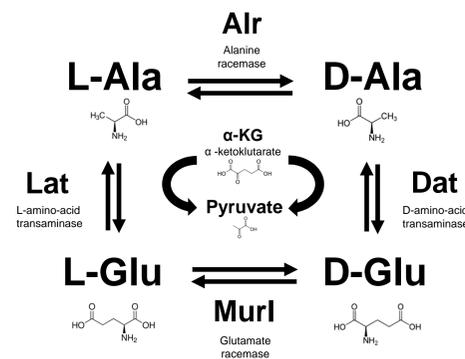
Introduction

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 *murl* genes encode essential functions. However, in studies performed by our lab, *alr* and *murl* 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 *murl* genes encode essential functions.

Background

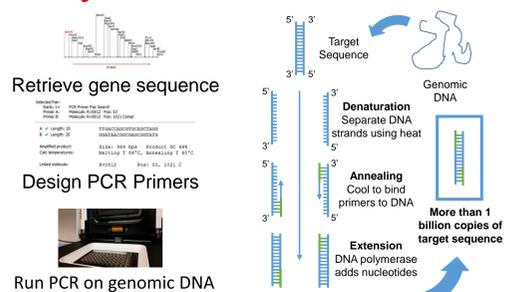
The Alanine and Glutamate Love-Square



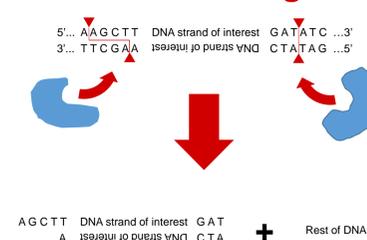
Glutamate and alanine are two vital amino acids for building peptidoglycan. In mycobacteria, the genes *alr*, *murl*, *lat*, and *dat* code for enzymes that can be used as shown above. We hypothesize that Lat and Dat, which have not been identified, provide redundant function in the absence of mutations in *alr* and *murl*. 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



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 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.

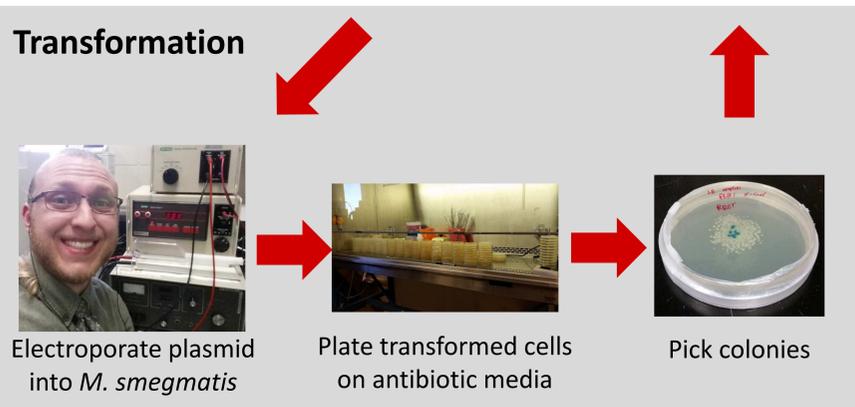
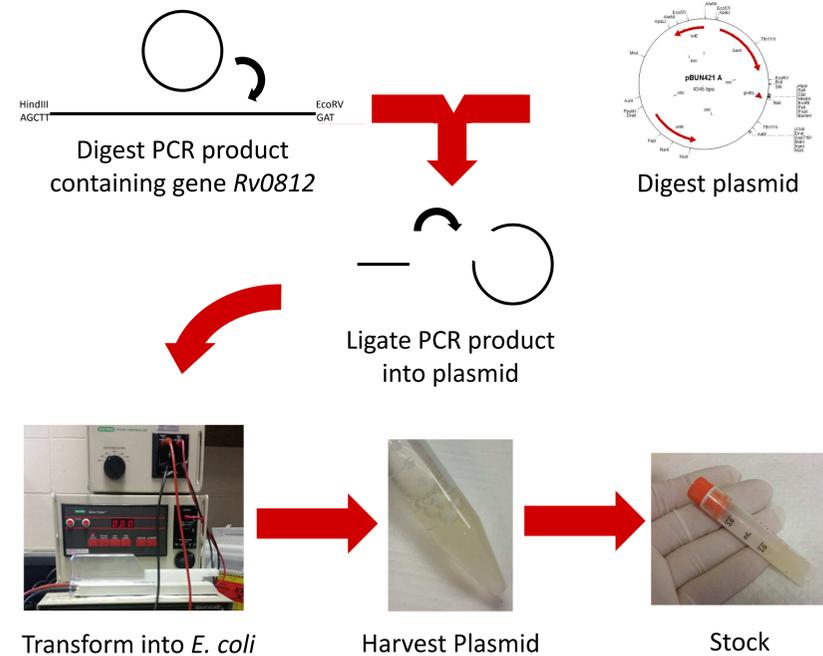
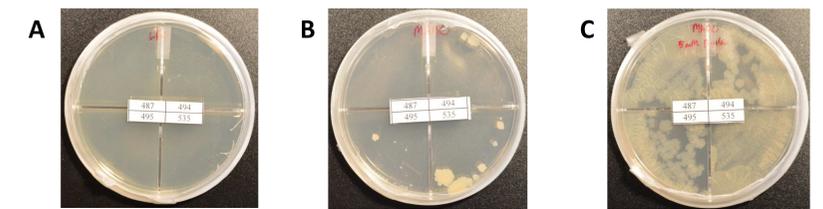


Fig 2. Growth analysis of multiple plasmids in *M. smegmatis*



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 M_{smeg_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 *murl* 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

This project was supported by grants from the University of Nebraska – Lincoln Undergraduate Creative Activity and Research (UCARE) program, the National Institute of Health (NIH) with an Exploratory/Developmental Research Grant Award to Dr. O. Chacon (R-21), the UNL School of Veterinary Medicine and Biomedical Science, and the Nebraska Agricultural Experiment Station Interdisciplinary Research Program and Animal Health funds.

We would like to thank Govardhan Rathnaiah for technical advice and assistance.