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Investigating Mycobacterium smegmstis' Electron Transport Chain Through Use of Chemical Inhibitors

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Investigating *Mycobacterium smegmatis*' Electron Transport Chain Through Use of Chemical Inhibitors

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Mycobacterium tuberculosis has infected approximately one out of every four people globally as reported by the CDC. One possible source for an antibiotic solution is the Electron Transport Chain. Using *M. smegmatis* as a model organism, I investigate the mycobacterial electron transport chain using known chemical inhibitors and identifying whether they have a specific target within the electron transport chain. Two oxidoreductases of interest include *qcrB* and *cydA*. My current research involves work with the CWHM1023 (CB81-family) chemical inhibitor. It is hypothesized that this compound acts through targeted inhibition of the *qcrB* oxidoreductase. This hypothesis is based on prior experiments showing a decrease in ATP levels of *M. smegmatis* following exposure to CWHM1023, as well as the discovery of a mutation in *qcrB*, $QcrB^{A178T}$, conferring resistance to CWHM1023. In an effort to confirm this hypothesis, various deletion mutants of *qcrB* and *cydA* in *M. smegmatis* have been constructed using novel phage recombineering methods. These mutants are then complemented with either empty vector, the original deletion, or a mutant version of the deleted sequence ($QcrB^{A178T}$) in an effort to confirm with certainty the targets of CWHM1023. To test the effects of CWHM1023 on the various strains, I utilize the Microplate Alamar Blue Assay (MABA), which tests for respiration levels of bacteria through quantification of the reduction of blue-colored Resazurin to the pink Resorufin. Numerous MABA experiments have thus far been conducted, and have provided promising data supporting *qcrB* as the target of CWHM1023. However, current research directions involve cloning of new complement strains that account for possible genetic polar effects given the existence of *qcrB* and *CydA* in operons. Confirmation of *qcrB* as the target of CWHM1023 will provide novel understanding of the organization of the mycobacterial electron transport chain, and ways by which it may be inhibited.