436q Using Inverse Metabolic Engineering to Restore Antibiotic Sensitivity in a Resistant P. Aeruginosa Isolate

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Antibiotic resistance is a pervasive and growing clinical problem. Bacterial infections that prove difficult to cure are often treated with combination therapies that simultaneously increase susceptibility and target essential genes. Our aims are to improve understanding and examine the potential for using combinatorial therapies against resistant infections. Towards this end, inverse metabolic engineering tools were employed to create and characterized mutants displaying aminoglycoside-sensitive phenotypes from a multi-drug resistant isolate of *P. aeruginosa*. Following random chemical mutageneisis, the frequency of finding mutants with heightened sensitivity levels was 10^{-1}, several orders of magnitude greater than frequencies reported for identifying mutations that increase resistance ($10^{-5\text{-}10}$). Transcriptional profiles using Affymetrix GeneChips were obtained from the resistant isolate, two sensitive mutants, as well from the sequenced laboratory strain, PAO1. Hierarchical clustering and principle component analysis revealed that transcriptional profiles of the sensitive mutants more closely resembled the sensitive laboratory strain PAO1 and each other than the parental resistant isolate. In particular, genes related to cell permeability and aminoglycoside-modification had significant changes in expression levels between the resistant isolate and the sensitive mutants. Minimum inhibitory concentrations performed on spheroplasts suggested that outer membrane permeability contributed significantly to the noted changes in resistance levels. Altered patterns of resistance to multiple aminoglycosides suggest that activity of aminoglycoside-modifying enzymes. Our results indicate that there are a large number of ways in which sensitivity could be restored to resistant isolates and that there is large potential for the use of combination therapies in the treatment of resistant infections. We are currently working on the construction of genomic libraries that will be screened for genes conferring amikacin resistance as well as a promoter probe with both negative and positive selection genes that will be used for identifying genes responding to antibiotics.