A PRELIMINARY STUDY ON ESSENTIALITY OF DIHYDROXYACETONE KINASE IN METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) is the leading cause of nosocomial infections in the United States and is resistant to multiple antibiotics. Our previous *in silico* analysis (Baye, N., K. Velk and C. Wu. 2009. Genomics approach for the identification of drug targets in Methicillin/Multiple-Resistant *Staphylococcus aureus* (MRSA). Proceedings of the South Dakota Academy of Science 88:202.) identified 126 and 132 potential drug targets in MRSA 252 and MRSA Mu50 strains respectively that are essential to pathogen survival and absence in the human genome. In this research, we examined the preliminary work of using an allelic replacement approach (Manna A. C., and A. L. Cheung. 2006. Expression of SarX, a negative regulator of agr and exoprotein synthesis, is activated by MgrA in Staphylococcus aureus. J. Bacteriology 188:4288-4299.) to validate the essentiality of one target, MRSA dihydroxyacetone kinase (DAK). A DNA fragment containing 1 kb upstream and 1 kb downstream region of the dak gene was cloned into a plasmid pCR 2.1 TOPO-TA easy vector system. An erythromycin sensitive gene was inserted into the recombinant pCR 2.1 plasmid to silence the dak gene. Our future work includes a subclone of the resulting silent gene into a pCL52.2, a temperature sensitive *E. coli–Staphylococcus aureus* shuttle vector, transformation of recombinant pCL52.2 into *Staphylococcus aureus* RN4220 strain then into clinical strains and induction of homologous recombination in *Staphylococcus aureus* chromosomes.