Suppression of Antimicrobial Resistance Using CRISPRs

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Plasmids containing a catalytically dead Cas9 endonuclease (dCas9) system were studied as a potential repressor to the mecA methicillin resistance gene in MRSA. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) are genetic elements that function with CRISPR-Associated (Cas) proteins as adaptive immune systems in bacteria. This CRISPR-Cas system acts as a targeted endonuclease that destroys the DNA of invading bacterial pathogens. Methicillin-resistant Staphylococcus aureus (MRSA) is a common bacterium responsible for many human maladies and is especially dangerous due to its resistance to antibiotics. The reference sequence of mecA from the MRSA strain ATCC 43300 was used to look for potential CRISPR target sites. Sites were evaluated based on their location to the gene promoter and the presence of Protospacer Adjacent Motifs (PAMs), sequences essential for target recognition. Two target sites were chosen and engineered into plasmids containing chloramphenicol resistance genes for selection purposes. Specialized Escherichia coli (E. coli) and MRSA cells were made electrocompetent using a protocol specific to experiments using the specialized cells. A plasmid for each site and a control plasmid were electroporated into E. coli and then grown overnight on agar plates with chloramphenicol to select bacteria that had accepted the plasmid successfully. Plasmids were isolated using a standard extraction kit and electroporated into competent MRSA cells. When plated on chloramphenicol agar plates, the MRSA did not grow. Future research will make further attempts to electroporate MRSA at higher voltages.