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In many cases, Reactive Oxygen Species (ROS) are known to be generated in bacterial cells in response to antibiotics. As increasing resistance among pathogens has rendered antibiotics and antifungals less and less effective, it is imperative to test for novel compounds. The goal of this project is to develop a bio-sensor that can detect ROS production by bacteria in the presence of antibiotics. This bio-sensor can then be used to identify novel antibiotics and antifungals.

We have modified a yeast enhanced Green Fluorescent Protein (yGFP) to be ROS sensitive. Using site directed mutagenesis, we introduced point mutations S147C and Q204C to thus allow us to discern in vitro antibiotic activity by measuring the levels of ROS. RoyGFP (reactive oxygen sensitive yeast enhanced GFP) was inserted into cells to enable us to compare the minimum concentration to generate ROS with the MIC. Since hydrogen peroxide mimics the effects of ROS, we added various concentrations of hydrogen peroxide to *E. coli* cells with our modified GFP plasmid. Our results showed that 0.5mM of hydrogen peroxide yielded the strongest ratiometric difference between the wild-type GFP and the royGFP. Various antibiotics such as Kanamycin and Norfloxacin will be tested on *E. coli* antifungals such as Fluoconazle and Ketoconazole will be tested on *Candida albicans* using this method. *E. coli* cells will be tested first to ensure the protocol is functional. I will be generating a BFP cell called azurite using the mutations T65S and V244R, as the final plan will be to be able to target various organelles and their localization signals in fungi with corresponding fluorescent proteins to be able to isolate and monitor their ROS generation. The ensuing results could not only lead to a novel technique to test antibiotics and antifungals, but it could also shine a new light on the development of new compounds.