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Johnathan Wong
Louisiana Tech University

Patrick L. Hindmarsh
Louisiana Tech University

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Testing ROS Sensitive Green Fluorescent Protein Using Secondary Metabolic Products From Different Species of Bacteria

Johnathon Wong¹, Patrick L. Hindmarsh²

¹*Undergraduate Student, School of Biological Sciences, Louisiana Tech University*

²*Associate Professor, School of Biological Sciences, Louisiana Tech University*

Reactive Oxygen Species (ROS) are known to be a natural byproduct of metabolism and are generated in response to certain antibiotics. The ultimate goal of this project is to develop a bio-sensor that can detect ROS production by bacteria in the presence of antibiotics. I am currently investigating whether spent media generates ROS. Competition between microorganisms for various resources such as nutrients and space has been well documented. Whether it is in different bacterial species or in bacteria or eukaryotic cells, this competition is pervasive. One method in which these cells compete is through the secretion of secondary metabolites that can either inhibit the growth or kill target cells. These metabolites are dispersed in the cell media and can be isolated through centrifugation and filtration of the supernatant. The media that contains these metabolites but not the cells themselves is known as spent or conditioned media. Prior investigations suggest that this spent media can potentially impede cell growth. I am interested in whether spent media from specific species of bacteria can inhibit bacteria and if so, if these effects can be detected. We have modified a yeast enhanced Green Fluorescent Protein (yeGFP) 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. I am currently investigating the ROS production by using spent media, or media that has had its nutrients used up by specific species of bacteria such as *B. cereus*, *S. marcescens*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* generates in *E. coli* cells as a potential method to screen antibiotics that do not naturally induce stress. Applying this novel technique, we ultimately plan to develop a small molecular library and thus shine a new light on the development of new antibiotics