Microorganisms are capable of producing advanced biofuels that can be used as 'drop-in' alternatives to conventional liquid fuels. However, the cell machinery of these microorganisms often becomes overwhelmed by the toxic effects of the biofuel product. In order to make microbial biofuels a competitive fuel source, mechanisms of improving resistance to the toxic effects of biofuel production is vital.

Our investigation aims to identify resistance mechanisms from microorganisms that have evolved to withstand extreme environmental pressures. Using a plasmid-based transgenic library approach, genes believed to impart resistance can be inserted and studied in a different microorganism. Adaptions such as increased efflux pump efficiency and less permeable cell membranes could improve biofuel tolerance in the host organism, *Escherichia coli.*

Psedomonas aeruginosa was studied because the bacterium has evolved mechanisms to survive attacks from many damaging compounds. A plasmid library from *P. aeruginosa* was created and transformed into *E. coli.* The resulting cells were then stressed with biofuel to determine if any of the genetically altered *E. coli* displayed improved tolerance. Our studies identified specific genes from *P. aeruginosa* that significantly improve tolerance to limonene when expressed constitutively in *E. coli*. Using the transgenic library approach, other microorganisms with interesting tolerance mechanisms can also be studied.