CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Rohini Shantharam

Project Number
S0424

Project Title
Searching for Mechanisms for Activating 2,4-D Degradation in Soil Bacteria TFT5, TFT39, K19: A Comparative Promoter Stud

Abstract

Objectives/Goals
2,4-Dichlorophenoxyacetic acid is a recalcitrant xenobiotic herbicide widely used as broad-leaf weed control for cereal crop production. The toxin has been accumulating in our soil thus increasing likelihood of health risks associated with its exposure. Studies have shown that the plasmid pJP4 from Ralstonia eutropha and Burkholderia spp. have evolved metabolic pathways to use 2,4-D as its soul carbon and energy source.
The main objective of this new approach is to test the promoters from JMP134 and RASC in various 2,4-D degrading bacterial soil strains to identify which promoter influences most enzyme activity. Prior to beginning research it was hypothesized that each promoter in each soil strain would show similar enzyme activity.

Methods/Materials
The experiment used E. coli containing plasmids with a variety of tfd promoters for the lacZ gene coding for beta-galactosidase. A quantifiable beta-galactosidase expression would be helpful for a better determination of promoter suitability. The experiment included mating, through conjugation, the E. coli containing cloned promoter regions from strain JMP134 and RASC with three different soil bacteria. Following this Beta-Galactosidase Assays were performed to quantify gene expression. Finally, DNA extraction and gel electrophoresis were run for further assurance of the plasmid in the soil strain.

Results
Results indicated that promoter pMD96 showed comparatively higher enzyme activity in both of the tested soil strains. Beta-Galactosidase Assays were terminated due to continuous growth of E coli. Gel electrophoresis indicated that promoter insert pMM7700 was deleted.

Conclusions/Discussion
This suggests that perhaps promoter pMD96 contains regulatory elements superior to the others. Understanding these gene regulatory mechanisms now will permit upcoming attempts at engineering toxin degrading regulatory and structural genes.

Summary Statement
The project tests various promoters from 2,4-D degrading soil strains TFT5, TFT39, and K19 to see which one results in more enzyme activity.

Help Received
The project was conducted at California State University of Fresno