Abstract

Bacteria have evolved systems for heavy metal resistance and reduction that can be exploited for bioremediation. This pilot research project looks at the reduction of chromium present at DOE’s Savannah River Site and the ability of the bacterial population to aid in the reduction of heavy metals. The research goal was to determine the genes responsible for chromium resistance in a Pseudomonas fluorescens Pf5 isolate capable of Cr(VI) reduction using transposon mutagenesis. P. fluorescens was mated with E. coli DH10B containing λpir which has a mini-Tn5 transposon. The conjugates were plated on Luria Bertani agar with kanamycin. Individual colonies were then replica plated onto LB agar with 25 ppm of potassium dichromate. One mutant out of 8000 mutants was found to have increased sensitivity to Cr(VI). The mutant will be examined for chromium reduction and the mutated gene will be determined.

Introduction

Bioremediation is a process that uses biological organisms to solve an environmental problem such as the removal of toxic waste at a contaminated site. This process enhances the growth of microbes at a contaminated site or microbes can be added to degrade the contaminants. It also enhances the biodegradation processes that occur in nature. Chromium is the target element for bioremediation in this research. Chromium is used in making steel and other alloys, and occurs naturally in volcanic soil, dust, plants, animals, water and roots. Of the two forms of chromium that can be found in nature, Cr(III) is an essential nutrient for humans that can be found in vitamins and various foods, and Cr(VI) is highly toxic and can cause various health effects. Pseudomonas fluorescens is a versatile species metabolically and can inhabit many environments. P. fluorescens is beneficial to both plant and human species. In plants, they reside around the roots where they have the capability to receive the nutrients they need to grow while also protecting the plant from infection. They protect the plant from infection by producing an antibiotic, and this antibiotic can also be used for treating bacterial skin infections in humans. The bacteria are easy to culture, and they grow quickly. P. fluorescens strain Pf5 can reduce Cr(VI) to Cr(III). The goal of this project is to determine the Pf5 genes involved in Cr(VI) reduction.

Materials and Methods

Plasmid DNA Isolation and Quick Transformation

E. coli DH10B λpir containing the transposon plasmid pTnModRKm and E. coli BW20767 (λ pir) were cultured on LB/kanamycin agar. pTnModRKm was isolated from a broth culture of E. coli DH10B λpir. A colony of E. coli BW20767 (λ pir) was transferred to a microscope containing transformation competence buffer and the tube was placed on ice. The contents of the tubes were then sonicated and returned to ice. After this, 5 µL of plasmid DNA solution was added and the tubes were plated back on ice for 5 minutes. Next the tubes were heated shocked and SOC broth and dyes were added. The tubes were then incubated at 37°C for 4 hours. Lastly, 100 µL of the culture was spread across an LB/DAP/kanamycin plate and placed back in the 37°C incubator for 24 hours.

Transposon Mutagenesis

This process consisted of three separate parts. E. coli BW20767 with pTnModRKm and P. fluorescens strains were spreaded for isolation from freezer stocks and the E. coli was incubated overnight in the 37 °C incubator and the P. fluorescens in the 25 °C incubator for two days. After incubation, overnight cultures were prepared for both of the strains. The next part of transposon mutagenesis was a conjugation assay. With this part, 100 µL of both the E. coli and the P. fluorescens were plated in a single microcentrifuge tube and then centrifuged at 6,000 rpm for 1 minute. The supernatant was removed and the mixture was resuspended and placed on the center of an LB/DAP plate. Lastly, the mixture was allowed to dry and was placed in the 25 °C incubator overnight. The last part in this process was the plating of conjugates. Next, 400 µL of LB broth was transferred to a microcentrifuge tube and all of the bacteria from the LB/DAP plate were added to the broth. Then, 600 µL LB broth was added and gently mixed with a pipette. Lastly, 100 µL of the bacterial suspension was spread over the surface of each LB/kanamycin plate and each plate was placed in the 25 °C incubator for two days.

Screening of Mutants with Increased Sensitivity to Cr(VI)

First, media was prepared with the appropriate concentrations of Cr(VI) and kanamycin. Then, replica plating of the conjugates was performed. After observing the results of the replica plating, two mutants that appeared to be sensitive to Cr(VI). These mutants were isolated and retested to confirm that they were sensitive. After the test, only one was shown to be sensitive. Lastly, a colony of this mutant was cultured and a freeze stock of the mutant was created.

Results

There were approximately 8,000 total mutants screened. A large number was screened to increase the probability of finding Cr(VI) sensitive mutants. Of the mutants that were screened there was one mutant that was sensitive. This mutant was cultured and a freezer stock was made for future use.

Future Plans

To conduct a chromium reduction assay with the chromium sensitive mutant that was cultured and made into a freezer stock.

References


Acknowledgements

• Claflin University Department of Natural Sciences and Mathematics
• Claflin University Department of Biology
• Department of Energy, Office of Environmental Management (DOE-EM000479)