Cr^{6+} sensitivity of *Pseudomonas fluorescens* transposon mutants

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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 *Escherichia coli* containing pTnModRKm' which has a mini-Tn5 transposon. The exconjugates 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^{6+}. 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 work 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 dust, soil, plants, animals, water and rocks. 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 to treat 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 DH5α λ pir containing the transposon plasmid pTnModRKm ' and *E. coli* BW20767 (λ pir) were cultured in on LB/kanamycin agar. pTnModRKm 'was isolated from a broth culture of *E. coli* DH5α λ pir. A colony of *E. coli* BW20767 (λ pir) was transferred to a microtube containing transformation competence buffer and the tube was placed on ice. The contents of the tubes were then vortexed and returned to ice. After this, 5 µL of plasmid DNA solution was added and the tubes were placed back on ice for 5 minutes. Next the tubes were heat shocked and SOC broth and diaminopimelic acid (DAP) were added. The tubes were then incubated at 37°C for an hour. 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 streaked 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 the both the *E. coli* and the *P. fluorescens* were placed in a single microcentrifuge tube and then centrifuged at 6,000 rpm for a 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 exconjugates. 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^{6+}

First, media was prepared with the appropriate concentrations of Cr^{6+} and kanamycin. Then, replica plating of the exconjugates was performed. After observing the results of the replica plating, two mutants that appeared to be sensitive to Cr^{6+}. These mutants were isolated and retested to confirm that they were sensitive. After the retest, only one was shown to be sensitive. Lastly, a colony of this mutant was cultured and a freezer stock of the mutant was created.

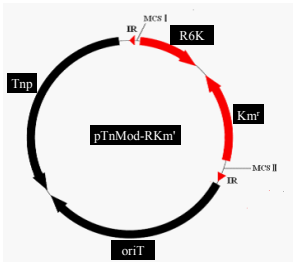
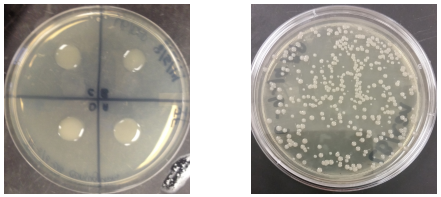


Figure 1: Map of pTnMod-RKm' plasmid that was transformed into *E. coli* BW29467. The transposon contains inverted repeats, a replication origin, and the gene for kanamycin resistance. The transposase gene and origin of transfer are outside of the transposon on the plasmid.

Results

Figure 2: Conjugation and Exconjugate Plating



E. coli BW29467 with of pTnMod-RKm' and *P. fluorescens* Pf5 were mixed, pelleted, resuspended in LB broth, and spotted onto LB/DAP agar. The mixture was recovered from the plate, placed in broth, and spread over LB/kanamycin agar. Colonies indicate transposon mutants.

Figure 3: Replica Plating

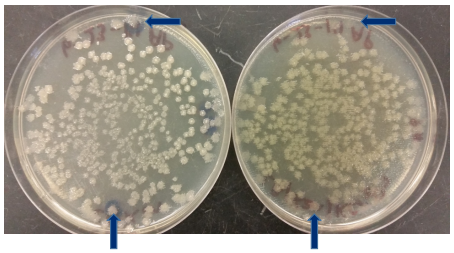
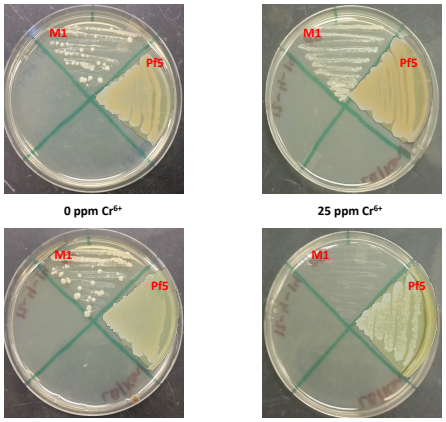


Figure 3. The arrows show where mutants were transferred but did not grow or grew poorly. This indicates that it was sensitive to Cr^{6+}.

Figure 4. Growth of *P. fluorescens* and transposon mutant



0 ppm Cr^{6+} **25 ppm Cr^{6+}**

50 ppm Cr^{6+} **100 ppm Cr^{6+}**

Figure 4. Pf5 and the mutant were cultured on separate media and the images were merged.

Conclusion

There were approximately 8,000 total mutants screened. A large number was screened to increase the probability of finding Cr^{6+} 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

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Acknowledgements

- Claflin University Department of Natural Sciences and Mathematics
- Claflin University Department of Biology
- Department of Energy, Office of Environmental Management (DOE-EM0000479)