Transposon Mutagenesis of *Stenotrophomonas Maltophilia* Oak Ridge strain 02

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Abstract

A multi-metal resistant strain of *Stenotrophomonas maltophilia* OR02 (*S. maltophilia* 02) was isolated from a metal contaminated site in Oak Ridge, TN. An EZ-Tn5 transposome was introduced into *S. maltophilia* 02. Approximately 880 transformants were replica plated onto plates containing copper sulfate, sodium selenite, mercuric chloride, sodium arsenite, and M-9 minimal salts medium to see if the transposon interrupted genes required for selenite resistance, arsenite resistance, mercury resistance, chromate resistance or growth on minimal media. 13 mutants were discovered. Of the 13 mutants discovered, 5 failed to grow on minimal media, 1 failed to grow on selenite, 1 failed grow on copper, 1 failed to grow on zinc, and 5 failed to grow on 2 or more metals. The transposon contains a kanamycin resistance genes and an *R6Kγ* replication origin. The genomic DNA from the mutants was purified, digested, ligated and transformed into *E. coli*. These transformants will contain new plasmids consisting of the transposon flanked by the interrupted genes. We expect DNA sequencing to identify genes involved in oxidative stress response, metal efflux, metal transformation (reduction and oxidation) and sequestration.

Introduction

The Y-12 plant at Oakridge, Tennessee played a vital role in the production of nuclear weapons processing uranium during World War II to make atomic bombs. During the Cold War in the 1950s, the Y-12 plant focused on processing lithium to make hydrogen bombs. In this process, a large quantity of elemental mercury was used of which 330 tons of mercury was lost to the environment contaminating the nearby East Fork Poplar Creek (EFPC) at the Y-12 plant[1,2].

Stenotrophomonas maltophilia ORO2 (*S. maltophilia* O2) is a gram negative bacterium, isolated from East Fork Poplar Creek and is resistant to metal salts of mercury, cadmium, copper, zinc gold and selenium[3]. Our goal is to study the metal resistance genes that are involved in the resistance mechanisms. For this purpose transposon mutagenesis of *S. maltophilia* O2 was used to identify genes that encode resistance to selenite and gold and that allow it to grow in M-9 minimal medium. Some of these interrupted genes were identified using a gene rescue technique.

Materials and Methods

- *Transposon Mutagenesis*[4]
  - -Transposon – DNA segments which transfers itself from one part of the genome to another part of that genome.
  - -EZ-Tn5 transposome from Epicentre Biotechnologies was introduced into *S. maltophilia* 02 by electroporation - (Figure 1).
- -Cells were spread onto kanamycin plates (Figure 3).
- -Colonies that grew should have transposon inserts.
  - -The colonies were replica plated and screened for metal sensitivity.
- -Confirmed mutants are recorded in Table 1.

Gene Rescue

- The digested DNA was ligated with T4 DNA ligase which circularized the digested DNA strands.
- The ligated DNA was transformed into *E. coli* and spread on kanamycin plates.
- Only circularized DNA fragments containing the transposon will grow.
- DNA was purified from the selenite, and other metal sensitive colonies.
- The purified DNA was partially digested using *BfuC* I

Figure 1:Transposon Mutagenesis

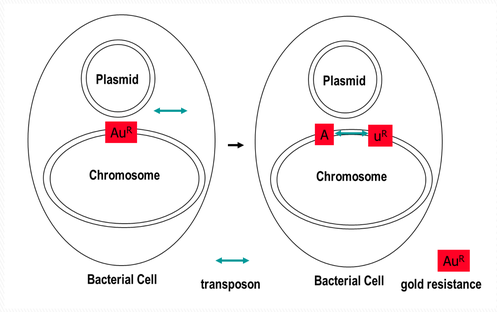


Figure 2: Replica Plating

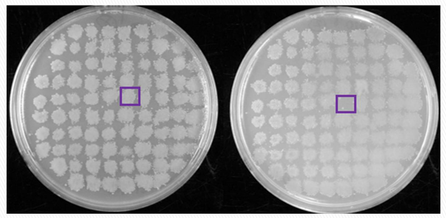


Figure 3: Partial Digestion of Genomic DNA

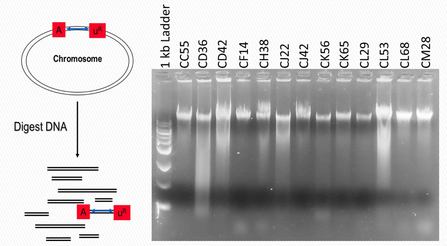


Figure 4: Recombinant Plasmids

Mutagenesis Results

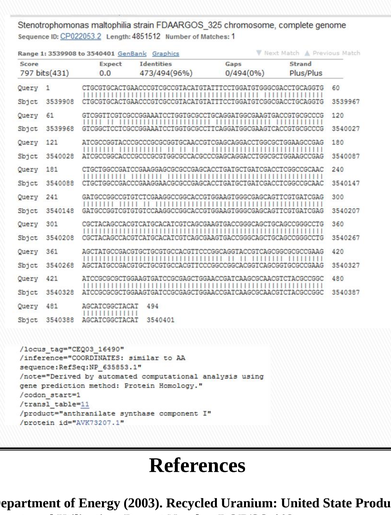
Mutant	LB-Kan	M-9	Se	Cu	Au	Zn	Cr	Mutated Gene
CD36	+	+	-	+	-	+	+	No Plasmid Discovered
CD42	+	+	-	+	+	+	+	No Plasmid Discovered
CF14	+	-	+	+	+	+	+	Anthranilate Synthase
CG55	+	+	+	+	+	-	+	Transporter
CH38	+	-	+	+	+	+	+	No Plasmid Discovered
CJ22	+	+	+	+	-	+	+	Sensor – his Kinase
CJ47	+	-	+	+	+	+	+	Shikimate Dehydrog
CK56	+	+	-	-	-	+	-	Sensor Kinase
CK65	+	+	-	+	+	+	+	Outer Membrane Protein
CL29	+	-	+	+	+	+	+	Aspartate Aminotransferase
CL53	+	+	+	-	+	+	+	No Plasmid Discovered
CL68	+	+	-	-	+	+	-	Aminotransferase
CM28	+	-	+	+	+	+	+	No Plasmid Discovered

LB-Kan = LB + Kanamycin, M-9 = minimal medium + casamino acids, Se = 10 mM Se(IV), Cu = 2 mM Cu(II), Au = 25 μM Au(III), Zn = 1 mM Zn(II) , Cr = 0.2 mM = Cr(IV), + = growth, - = no growth

Sequencing and BLAST Analysis

CJ22

CF14



Conclusion

- The selenite-sensitive mutant, CK65, contained mutations in an outer membrane protein, OmpA, which may be involved in efflux.
- The gold sensitive mutant, CK56, contained a mutation in a gene for a Sensor Kinase protein.
- The mutant, CF14, failed to grow on minimal medium and contained a mutation in gene for anthranilate synthase, which is important for tryptophan biosynthesis.
- Not all sequencing reactions were successful.

Summary of Mutants

Mutant Types	Number
2 or More Metals	5
Selenite	1
Zinc	1
Copper	1
Minimal Medium	5

Future Work

- Obtain sequence data for other mutants
- Obtain the complete sequence for outer membrane protein
- Use complementation experiments to show that the mutated gene can restore resistance

References

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