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) grows when exposed to toxic salts of gold and selenite. An EZ Tn*5* 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, arsenite resistance or growth on minimal media.

12 mutants were discovered. Of the 12 mutants discovered, 6 failed to grow on arsenic and selenite, 3 failed to grow selenite, 1 failed grow on arsenic, 1 failed to grow on copper, selenite, and minimal medium, and 1 failed to grow on minimal medium. 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.

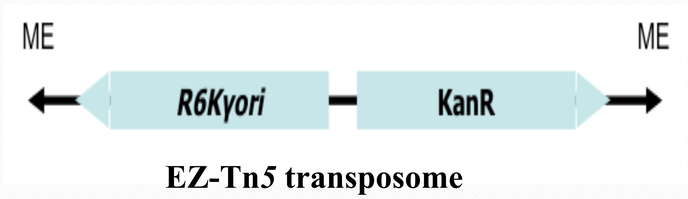
Materials and Methods

- *Transposon Mutagenesis*[4]
  - -Transposon – DNA segments which transfers itself from one part of the genome to another part of that genome.
  - -EZ-Tn*5* 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: Transposase Protein



EZ-Tn*5* transposome

Figure 2:Transposon Mutagenesis

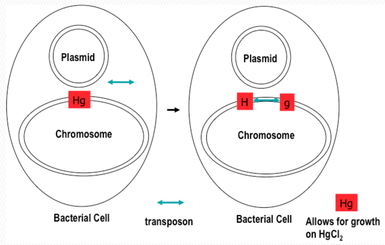


Figure 3: Kanamycin Plate Spread

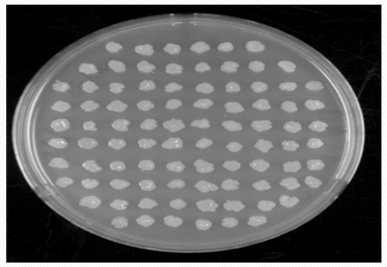
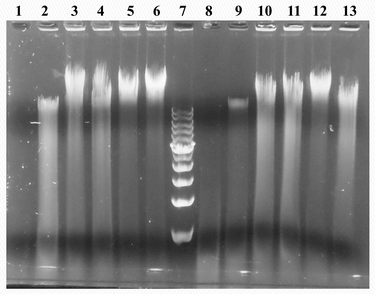


Figure 4: Partial Digestion of Genomic DNA

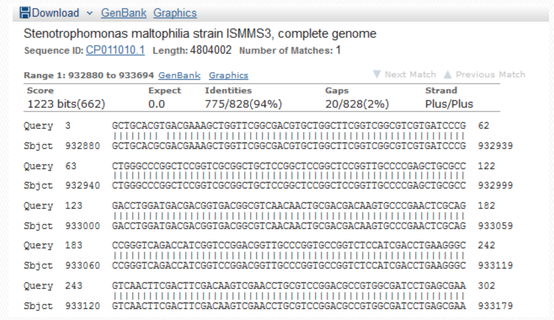


1. BT 54
2. BT67
3. BT94
4. BX 45
5. BY 26
6. BY80
7. 1 kb Ladder
8. BZ 9
9. BZ 20
10. CA 17
11. CA 18
12. CB 56
13. CC42

Mutagenesis Results

Mutant	LB-Kan	M-9-CAA	R3A-Se 10	R3A-Hg 25	R3A-Cu 1	R3A-As 5	LB-Kan
BT 54A	+++	+++	+	+++	+++	+++	+++
BT 67A	+++	+++	-	+++	+++	-	+++
BT 94A	+++	+++	-	+++	+++	+	+++
BX 45A	+++	-	-	+++	-	+++	+++
BY 25A	+++	+++	-	+++	+++	-	+++
BY 80A	+++	+++	-	+++	+++	+++	+++
BZ 9A	+++	+++	-	+++	+++	+	+++
BZ 20A	+++	-	+++	+++	+++	+++	+++
CA 17A	+++	+++	-	+++	+++	+	+++
CA 18A	+++	+++	-	+++	+++	-	+++

Sequencing and BLAST Analysis



Conclusion

- The selenite/arsenite-sensitive mutants, BT94, BY26, CA17 and CA18, contained mutations in an outer membrane protein, OmpA, which may be involved in efflux.
- The selenite sensitive mutant, CC42, contained a mutation in a gene for a regulatory protein.
- The mutant, BZ20, 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
Arsenic and Selenite	6
Selenite	3
Arsenic	1
Copper, Selenite, and Minimal Medium	1
Minimal Medium	1

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

1. US Department of Energy (2003). Recycled Uranium: United State Production, Enrichment, and Utilization. Report Number DOE/SO-003.
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4. CAGUIAT, J. (2014). Generation of *Enterobacter* sp. YSU Auxotrophs Using Transposon Mutagenesis, J. Vis. Exp. 92:e51934

Acknowledgement

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