Whole Genome Sequence and Development of a Transposon Mutagenesis System for *Pseudomonas Putida* sp.12

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Abstract

Nowadays, Heavy metal pollution is one of the most important environmental problems. All metals occurring in the biosphere originated in the earth's crust; however, the toxic metal pollution of the biosphere has intensified rapidly since the onset of the industrial revolution, posing major environmental and health problems. Several studies have demonstrated the effect of metals on microbial diversity, biomass and activity. It is believed that metal Microbial activity is responsible for the bioremediation of the third elements in the periodic table. Selenium is an important trace element that required for in trace amounts for growth and metabolism but toxic at elevated concentration. The ability of a single mutant *Pseudomonas putida* sp.12(mariner transposon) has been used to search for genes involved in the biotransformation of selenite to red elemental selenium. This study aims to develop a transposon mutagenesis system for the model *P. putida* sp.12 to investigate the gene(s) that involved in selenite reduction. The conjugative plasmid pSAM_R1 containing the mariner transposon was used for transposon mutagenesis. A single mutant reliably impaired in selenite transformation, found that the mutant clones, contained an inactivation within *pmoB*, one of two copies of the gene encoding the largest subunit of particulate selenite-reduction. Physiological analysis of this "white mutant" indicated that the selenite-reducing activity, which was located primarily in the cytoplasm of the cells, could be rescued by adding format as an alternative source of electrons.

Keywords: *Whole genome sequences, Selenium reducing bacteria; transposon; Bioremediation.*

Introduction

Microorganisms often keep metal haemostasis by pumping toxic metals out of cells using primary and secondary transporters [1].The best mechanism of avoiding an excess of metal ions is active transport by cation-transporting ATPases (Fig. 1). Selenium (Se) has both metallic and non metallic prosperities. It is an essential micronutrient for both prokaryotes and eukaryotesat low concentrations, but it can be toxic at higher levels. Se exists in nature in multiple organic and inorganic forms, Se can act as an antioxidant and protect against the cellular damage caused by oxygen radicals; however, an overdose of Se can disrupt the integrity of proteins and decrease the enzymatic activity, resulting in chronic or acute selenosis[2].

Many resistance determinants on chromosomes and mobile genetic elements encode a range of membrane transporters that transport specific toxic metals out of the cell. Some microorganisms use plasmid-encoded energy-dependent metal efflux systems to remove metals from the cell[3]. For instance, in response to exposition to toxic metals, metal resistance comes mostly in plasmid–encoded bacteria. Resistance genes encode genetic information of microorganisms that is charged by external or internal conditions[4].

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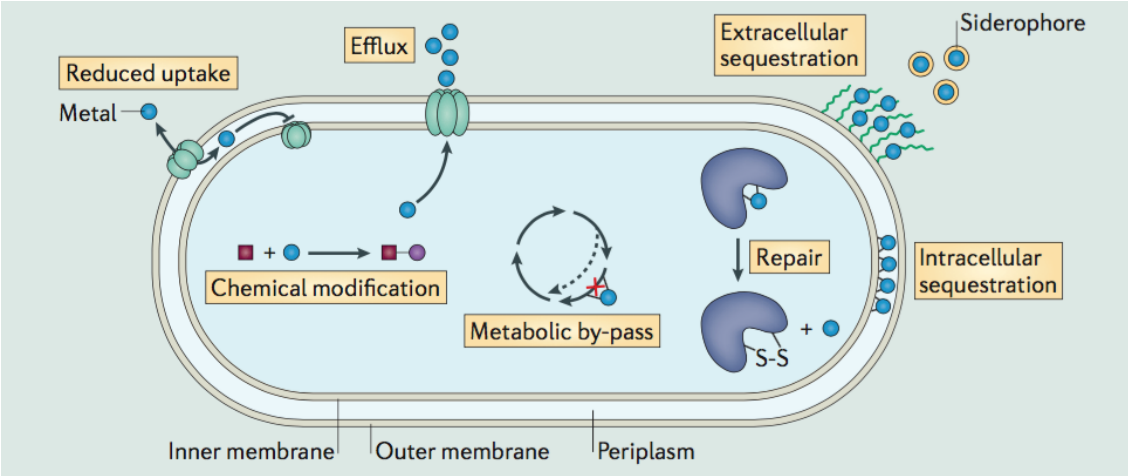

Figure 1. Main metal resistance mechanisms in bacteria[5].

Pseudomonas putida sp.genetic manipulation has proven a challenge because of the natural transformable. Electroporation of *P. putida* sp. has been found effective only with small plasmids[6]. There are slow growing means that some forms of antibiotic selection cannot be used and vectors used with *E. coli* are often not functional. An earlier work has shown that conjugation can be used to introduce plasmids into *P. putida* sp.12, and vectors have been developed to allow mutagenesis of the soluble methane monooxygenase (S) and heterologous protein expression. Because of *P.putida* sp.12 adaptability to toxic chemicals, the model soil bacterium *P. putida* sp. was developed via develop a transposon mutagenesis system for the model *P.putida*sp.and to use this to investigate the gene(s) involved in reduction and remediation of selenite to elemental selenium. The conjugative plasmid pSAM_R1 containing the mariner transposon, which was developed for transposon mutagenesis in the *Rhizobiaceae*[7]. This study aimed to was chosen to construct a transposon library to enable screening for mutants altered in specific metabolic functions including reduction of selenite.

Materials and Method

Bacterial strains and growth conditions: The *Pseudomonas putida sp.*12 was obtained from the biotechnology laboratory of Diyala University. The strain was growth routinely in LB medium. M9 minimal medium, amended with carbon source[8]. *P. putida* sp.12 was incubated at of 30 °C, while *E.coli* was grown at of 37°C.

The experiments were performed in 150 mL liquid cultures in 250 mL conical Quickfit® flasks cultures were incubated at the optimum growth temperature, on a shaker at 170 rpm, and allowed to grow to an OD_{600} of between 0.5-0.8. Under the conditions used in these experiments.

DNA techniques, plasmid concentrations and Conjugation process: To create the transposon library for *P. putida* sp.12, Plasmid pSAM_Rl has four unique restriction sites (*Nde*I, *Bam* HI, *Xba* I and *Xho* I) was obtained from *E. coli DH5*α using. The plasmid was extracted and digested using QIAGEN kit (Qiagen, MD, USA). Agarose gel analysis was used to confirm its integrity.

The conjugation was used following the method of Lloyd and co-workers[9]. The progeny from the conjugation were resistant to kanamycin (15 µg/ml). Subsequent plating on LB medium also containing nalidixic acid (25 µg/ml final concentration) was performed in order to eliminated the donor *E. coli* strain.

DNA Sequencing: The mutants strains were grown on the LB agar with kanamycin (15 µg/ml), and *P. putida* sp.12 grown on separate plates of the same medium without antibiotic was used as a control. Plates were incubated at 30°C. The bacteria were harvested by scraping from the plates and sent for sequences. To detect the number of inserted DNA segments and the transposon position, Mauve sequence analysis package and Mauve Contig Mover (MCM) were employed to re-order contigs for each new draft genome based on comparison to *P.putida*KT2440genome from the Genbank (accession number NZ_JBOP00000000) [10].

The position of the inserted transposon was detected in each contig and compared with the transposon sequence using BLAST to find the location of the transposon[11].

Characterization of mutant strains: The LB plates with 15 µg/ml final concentrations of kanamycin and 10 µg/ml of (Se selenite were used to re-grown white colonies carried the transposon library. The positive control was a kanamycin-resistant clone from the transposon library that gave red colonies on selenite-containing medium. The plates were incubated in 30°C for 2 4 hrs.

Results

To transfer pSAM_R1 into *P. putida* sp.12, the selected cells were plated on LB agar with kanamycin. Figure 2 confirmed the plasmid pSAM_R1 conjugation, presumably the conjugated plasmid showed antibiotic resistance; while the cells of *P. putida* sp.3 without conjugation (WT) did not grow and no colonies were observed (Fig 3). Around 500 colonies were obtained when the suspension (50 µl) of the conjugated cell was plated on the selective agar. However, non-conjugated cells were grown on medium without kanamycin which conformed strongly the conjugation theory of pSAM-R1 into *P. putida* sp.12 was successful.

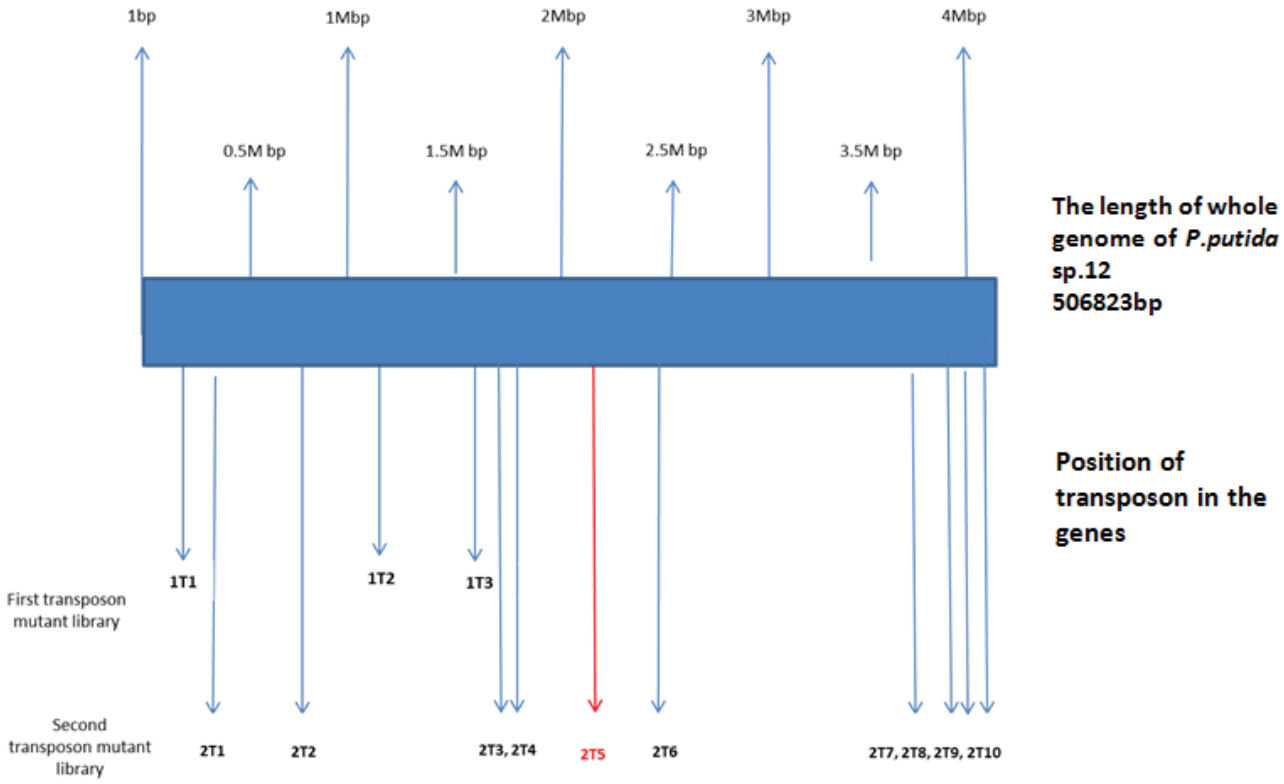

Figure 2. Positions of transposon insertions in the genome of *P.putida* sp.12

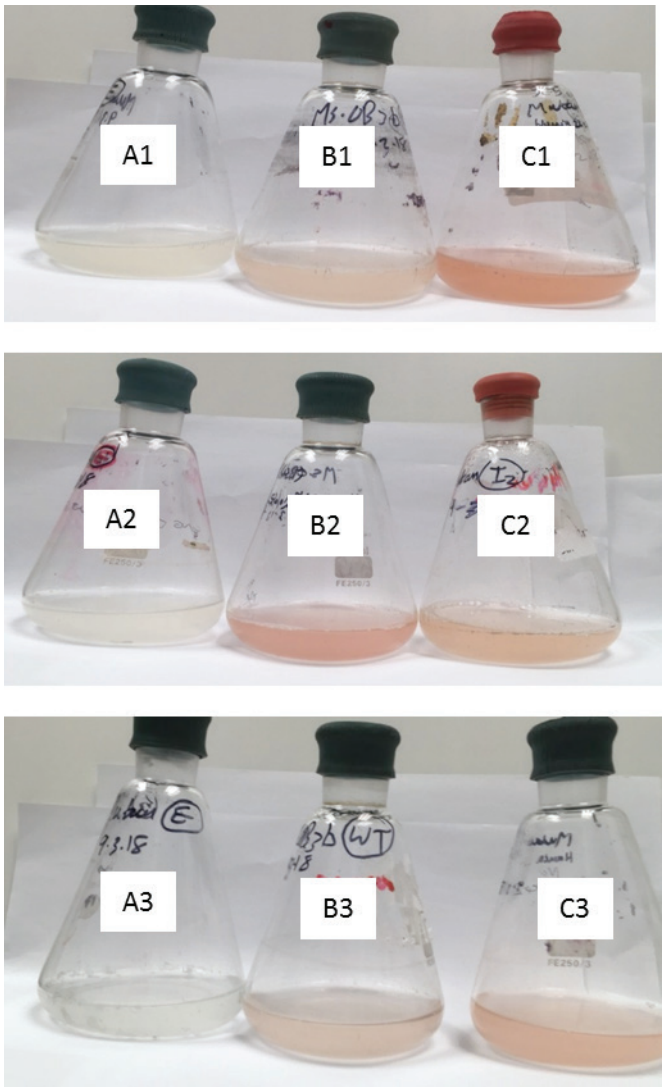

Figure 3: Triplicate experiment to show selenite reduction by the "white mutant" (*P. putida* sp.12) (A1, A2, A3), wild-type *P.putida.* sp.3 (B1, B2, B3) and the selenite reduction-positive ("red") mutant (C1, C2, C3) in LB liquid media containing selenite (containing 10 g/mL of Se) and grown on c as the carbon and energy source.

Whole Genome Sequencing: To check the position of the transposon on the genomic DNA, nine samples were prepared: eight putative transposon mutants and one samples of wild-type of *P. putida* sp.3. The subjected sequences comparison to the reference genome displayed additional segments indicated that there was a region within the genomes from the putative transposon mutants that displayed no similarity to the reference genome. The added segments were conserved among all putative mutant draft genomes but were not present in the genome data obtained in parallel for the wild-type strains.BLAST searches of the added DNA present in the chromosomes of the putative transposon mutants were performed. The confirmed that the extra DNA was the mariner transposon.

Gene involved in selenite reduction: The "white" transposon mutant 2T5 (which is inactivated in one of the two copies of *pmoB*) must still be able to grow using carbon as growth substrate and may be impaired in the supply of carbon-derived electrons for reduction of selenite. If this were correct, it may be possible to restore selenite reduction in the mutant by supplying format, which the carbon can use as an electron source via format dehydrogenase.

Discussion

This study used transposon mutagenesis to identify the role of *P. putida* sp. in the remediation of selenite $(SeO_3)^{2-}$.To screen very large number of transposon

insertion clones (≥100,000), well-developed genetic systems have recently been used to obtain high coverage of the selected genome[12]. The mutant colonies are presumably able to assemble fully functional pMMO because the strain retains the other copy of the pMMO operon[13]. Similarly, study aimed to transfer transposon IS-O-Km/hah from *E. coli* S17 to *Pseudomonas* strains Pph 1302A and 1448A to screen the progeny for their plant colonization ability[14]. Gene interruption by transposon in this mutant was one of the copies of *pmoB*, encoding the large subunit of pMMO[15]. However, in this study the appearance of selected colonies on LB agar plates could simply be the result of slow growth. Further analysis in liquid media after growth to OD_{600} with appropriate controls confirmed that the mutant was impaired in reduction of selenite. Earlier studies confirmed that the phenotype of mutantis not due to inactivation of an enzyme which is specifically involved in reducing selenite to elemental selenium, but due to a more general lesion in the metabolism of the methanotroph. When electrons were fed via another route from formate via formate dehydrogenase, reduction of selenite to red elemental selenium was restored, indicating that this route for feeding electrons into the reduction of selenite is also possible[16].To conclude, transposon mutagenesis by using mariner transposon in pSAM_R1 has been used to impair the gene that is involved in remediation of selenite $(SeO_3)^{2-}$.

Acknowledgement: Authors are thankful to authorities of Diyala University, College of Science for providing the necessary facilities to complete the work successfully.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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