

## RESEARCH NOTE

# TN5-OT182 SHOULD NOT BE USED TO IDENTIFY GENES INVOLVED IN BIOFILM FORMATION IN *BURKHOLDERIA PSEUDOMALLEI*

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**Abstract.** *Burkholderia pseudomallei*, a gram-negative bacterium, is the causative agent of melioidosis. One of the important virulence properties of this bacteria is its ability to form a biofilm. Genes involved in biofilm formation in *B. pseudomallei* have not been thoroughly studied. In this study, Tn5-OT182 mutagenesis was used to isolate of *B. pseudomallei* strain A2 mutants unable to produce biofilm. Ten biofilm-defective transposon mutants were isolated and analyzed. Flanking DNA from each transposon mutant were self-cloned and sequenced, then the sequences were analyzed with the BLAST program. To confirm these genes are involved in biofilm formation, we constructed three gene deletion mutants marked with a tetracycline resistance gene. The constructed *tet<sup>r</sup>*-marked deletion mutants were checked for correct structure and size by polymerase chain reaction. When subjected to biofilm assay, all tested *tet<sup>r</sup>*-marked deletion mutants were still able to produce biofilm, indicating the three genes are not involved in biofilm formation. These results suggest integration of Tn5-OT182 in genes not involved in biofilm production can render *B. pseudomallei* unable to produce biofilm by an unknown mechanism. This information demonstrates Tn5-OT182 is not a reliable tool for identifying genes involved in biofilm formation unless a confirmatory experiment is carried out in parallel.

**Keywords:** *Burkholderia pseudomallei*, melioidosis, biofilm, Tn5-OT182, transposon

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