

TITLE: The *Burkholderia thailandensis* EstA-like protein is a putative autotransporter system

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ABSTRACT:

Burkholderia thailandensis (Bt) is a non-infectious species, commonly isolated from soil and water, that has been widely used as a surrogate model for studying many characteristics of its pathogenic counterparts within *Burkholderia spp.* Bt produces rhamnolipids, which is directly involved in multicellular behavior. The study of biosynthetic pathways of rhamnolipids in *Pseudomonas aeruginosa* revealed an autotransporter protein called EstA, which greatly influences the production of rhamnolipids, swarming motility, and the formation of adherent biofilms. EstA is characterized by a β -barrel motif in the C-region that anchors a passenger protein in the outer membrane and a lipase motive in the N-terminus. In order to investigate the presence and functionality of EstA-like autotransporters within rhamnolipid-producing *Burkholderia spp.*, bioinformatic tools were employed. The bioinformatic prospectations revealed a potential EstA-like autotransporter gene within the genome of Bt E264 (herein designated *orfE264*). Plasmid constructions containing deleted *orfE264* or *estA* were synthesized, followed by the insertion of appropriate drug resistance genes. *orfE264*-knockout Bt and *estA*-knockout *P. aeruginosa* were obtained by homologous recombination, using the three Lambda Red recombinases. Characterization of the resulting *orfE264*-mutant strain revealed significantly decreased biofilm formation and swarming motility, when compared to the wild-type strain, showing that this gene product is involved in those phenotypes. Interestingly, cross complementation of the *orfE264*-mutant with a full-length *estA* restored the phenotypes. In the same fashion, cross-complementation of the *estA*-mutant *P. aeruginosa* with *orfE264* has restored motility and biofilm formation. These results demonstrate the functionality of a novel autotransporter protein, which is the first member of this protein secretion family described in this species (Bt). The results obtained so far represent a great potential for clinical interests, since *B. thailandensis* is an important, non-pathogenic surrogate model for infectious *Burkholderia spp.* Moreover, the use of *B. thailandensis* for industrial production of rhamnolipids represents a great interest in biotechnology, and an EstA-like autotransporter may serve as a suitable target for metabolic engineering, aiming to reduce the production costs for this biosurfactant.

Keywords: *B. thailandensis*, *P. aeruginosa*, Biofilm, Autotransporter, EstA

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