Identification and validation of novel small proteins in Pseudomonas putida

Identification and validation of novel small proteins in *Pseudomonas putida*: Novel small proteins in *P. putida*

Small proteins of fifty amino acids or less have been understudied due to difficulties that impede their annotation and detection. In order to obtain information on small open reading frames (sORFs) in *P. putida*, bioinformatic and proteomic approaches were used to identify putative small open reading frames (sORFs) in the well-characterized strain KT2440. A plasmid-based system was established for sORF validation, enabling expression of C-terminal sequential peptide affinity (SPA) tagged variants and their detection via protein immunoblotting. Out of 22 tested putative sORFs, the expression of fourteen sORFs was confirmed, where all except one are novel. All of the validated sORFs except one are located adjacent to annotated genes on the same strand and three are in close proximity to genes with known functions. These include an ABC transporter operon and the two transcriptional regulators Fis and CysB involved in biofilm formation and cysteine biosynthesis, respectively. The work sheds light on the *P. putida* small proteome and small protein identification, a necessary first step towards gaining insights into their functions and possible evolutionary implications.

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