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Identification and validation of small proteins in *Pseudomonas putida* KT-2440

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Small proteins (s-proteins) that contain 50 or fewer amino acids have been overlooked due to difficulties in their annotation and identification. Recent research indicates that s-proteins widely exist in bacteria and are involved in various biological processes. Our research focuses on identification and validation of s-proteins in the production organism *Pseudomonas putida*. By using proteomics, bioinformatics tools and the *Pseudomonas* genome database (PGD), we have identified 283 putative small open reading frames (sORFs) in the *P. putida* KT2440 genome. The transcription, conservation and genome contexts of sORFs were analyzed and 32 conserved sORFs with evidence of transcription selected for validation. The sORFs are validated by expressing them with a C-terminal SPA-tag (Sequential peptide affinity tag) followed by Western blotting⁽²⁾. The expression of 3 sORFs has been confirmed and testing of 20 additional sORF candidates is currently in progress. This will be followed by functional characterization of selected sORFs.