

**TITLE:** Pangenome analysis and characterization of resistance and virulence genes in *Pseudomonas putida*

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**ABSTRACT:** *Pseudomonas putida* inhabit a wide range of environments. The capacity to promote plant growth, bioremediate toxic waste, and infect humans derive from the versatile and diverse genomes found across isolates. The genomes of several *P. putida* isolates are publicly available, providing a valuable resource for comparative genomics studies. A pangenome is the complete gene set of a given species and is divided into core and accessory genomes. Compared with *P. aeruginosa*, few studies have been conducted to explore its intra-specific variation. This study aimed to determine the pangenome of *P. putida* as well as its resistance and virulence profiles. To avoid misclassified genomes deposited in NCBI, we downloaded all 8,763 genomes from *Pseudomonas* genus and eliminated low-quality genomes based on BUSCO searches. We used *P. putida* KT2440 as a *type* strain to get genomes with more than 95% nucleotide identity. Pangenome categories were assessed using roary and SPNs were extracted to construct a maximum likelihood phylogenetic tree using RAxML. *P. putida* has an open pangenome comprising 27,849 gene families, demonstrating a high genetic diversity. The core-genome has 3,590 genes, indicating that all isolates in this study belong to the same species because of a high core-genome conservation. The phylogenetic analysis revealed that the *P. putida* population is composed by seven main phylogroups. The core-resistome lacks enzymes involved directly with  $\beta$ -lactam resistance, but the accessory genome harbors a range of beta-lactamases (eg. *bla*<sub>CARB-3</sub>, *bla*<sub>IMP-10</sub>, *bla*<sub>OXA-2</sub>, *bla*<sub>TEM-1</sub>, and *bla*<sub>VIM-2</sub>). The seven phylogroups are well defined based on their virulence gene profiles. Especially, the phylogroup containing *P. putida* BIRD-1 is defined by TonB-dependent siderophore receptors, which might confer an advantage for niche colonization because of the increased iron uptake capacity. Our results provide important information on the genetic diversity of *P. putida*, which will allow a more profound assessment of its safety to be used in biotechnological applications.

**Keywords:** Comparative genomics, population structure, resistome

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