



Complete Genome Sequence of a Plant-Derived Phenylpropanoid-Degrading Bacterium, *Pseudomonas putida* JYR-1

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ABSTRACT *Pseudomonas putida* JYR-1 was isolated from soil contaminated with industrial oil because of its ability to utilize *trans*-anethole as a carbon and energy source. The complete genome is 5.41 Mb with 4,834 protein-coding genes. Study of this isolate will provide insight into biotransformation pathways for phenylpropanoids.

Pseudomonas putida JYR-1 was isolated from soil contaminated with industrial oil in South Korea and is capable of degrading the plant-derived propylbenzene *trans*-anethole (1). Stanier's basal minimal salt buffer (MSB) containing 0.5% (wt/vol) *trans*-anethole was used as a selective medium during isolation. Soil suspensions were incubated with rotary shaking at 200 rpm at 27°C (1). The ability of isolated strain JYR-1 to transform phenylpropanoids, including *trans*-anethole, isoeugenol, isosafrole, and *O*-methyl isoeugenol, to the corresponding aromatic aldehyde has been characterized by expression of the gene encoding *trans*-anethole oxygenase in *Escherichia coli* (2). Further kinetic assays revealed *trans*-anethole oxygenase to be a novel self-sufficient monooxygenase that catalyzes the complete process of oxygenation and cofactor cycling in one subunit (3). In addition, *p*-anisaldehyde dehydrogenase was characterized to convert aromatic benzaldehydes such as *p*-anisaldehyde, vanillin, veratraldehyde, and piperonal to corresponding aromatic acids, *p*-anisic acid, vanillic acid, veratric acid, and piperonylic acid (4). A gene cluster involved in phenylpropanoid biometabolism was previously identified from a 34-kb genome fragment from *P. putida* JYR-1, which gave JYR-1 the potential to be an industrial strain for producing fragrances from plant-derived chemicals (2, 4). Thus, we sequenced the genome of *P. putida* JYR-1 in this study.

A cell culture of *P. putida* JYR-1 was grown in LB broth at 27°C and stored with 30% glycerol at –80°C after isolation. Prior to genome sequencing, this stock culture was streaked on LB agar. A single colony was picked and cultured in LB broth followed by cell collection. Genomic DNA of JYR-1 was extracted with the SDS method (5). The quality of the extracted DNA was assessed using agarose gel electrophoresis and quantified fluorometrically using a Qubit fluorometer. Unamplified libraries were prepared with the SMRTbell template prep kit 1.0 using the Pacific Biosciences standard protocol. Genome sequencing was performed using a PacBio RS II platform (6). In total, 80,142 high-quality subreads were filtered using the stand-alone PRINSEQ-lite version (7) with an average read length of 7,694 bp (N_{50} , 9,392 bp). *De novo* assembly was done using the Hierarchical Genome Assembly Process (HGAP) via SMRT Link 5.0.1 with two-round polishing using Quiver (8), generating a circular chromosome of

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5,413,503 bp with an overall G+C content of 62.48%. Default parameters were used for all software unless otherwise specified.

The genome was annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) (9) and Rapid Annotations using Subsystems Technology (RAST) 2.0 (10). We identified 4,834 protein-coding DNA sequences (CDSs) and 100 predicted RNAs (74 tRNAs, 22 rRNAs, and 4 noncoding RNAs [ncRNAs]). The public version of this genome in DDBJ/EMBL/GenBank (accession number [CP043835](https://doi.org/10.1093/nar/gkv1070)) was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) (11). In this study, annotations from KEGG were used to determine functional categories, and RAST and PGAP combined with previous biochemical investigations (2, 4) were used for interpreting the function of individual genes.

From the functional annotation of KEGG, 782 CDSs in the category “metabolism of terpenoids and polyketides” and 64 CDSs in the category “xenobiotic biodegradation and metabolism” were characterized. In addition to the *trans*-anethole oxygenase gene (*tao*), the *p*-anisaldehyde dehydrogenase gene (*paad*), and the vanillate monooxygenase genes (*vanA*, *vanB*) reported in a previous biochemical study of *Pseudomonas putida* JYR-1 (2), many other genes involved in degradation of aromatic compounds were found in the JYR-1 genome. Various catalytic functions for phenylpropanoid metabolism predicted in the *P. putida* JYR-1 genome indicate its potential as a tool for biosynthesis of aromatic chemicals from plant-derived materials.

Data availability. The complete genome sequence of *Pseudomonas putida* JYR-1 has been deposited in DDBJ/EMBL/GenBank under the accession number [CP043835](https://doi.org/10.1093/nar/gkv1070). The raw reads were deposited in the Sequence Read Archive (SRA) under the accession number [SRR10092016](https://doi.org/10.1093/nar/gky066).

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