Complete Genome Sequence of the Nicotine-Degrading *Pseudomonas putida* Strain S16

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***Pseudomonas putida* S16 is an efficient degrader of nicotine. The complete genome of strain S16 (5,984,790 bp in length) includes genes related to catabolism of aromatic and heterocyclic compounds. The genes of enzymes in the core genome and a genomic island encode the proteins responsible for nicotine catabolism.**

Pseudomonas putida is one of the best-studied species of the metabolically versatile and ubiquitous genus of *Pseudomonas*. These characteristics make *P. putida* strains attractive for environmental, biotechnological, and agricultural uses (3, 4, 8, 9, 12). Nicotine is very toxic and, with the rise in global tobacco consumption, it may be a leading toxicant, causing preventable disease and death. Various nicotine-degrading bacteria, including *Arthrobacter* and *Pseudomonas*, have been used in studies on biodegradation of this N-heterocyclic compound (1, 7, 14, 15). To date, none of the nicotine-degrading bacteria genomes has been fully sequenced to investigate the molecular mechanism. Here, we report the genome sequence of an efficient degrader of nicotine, *P. putida* S16 (13). The whole genome sequence will help to identify the genomic and metabolic diversity of this species (16).

The genome sequencing of *P. putida* S16 was performed with a combined strategy of Solexa paired-end sequencing (total reads, 25,595,310; 75 bp in each read) and Solexa mate-pair sequencing (total reads, 17,634,490; 36 bp in each read). The paired-end reads were assembled to contigs with the Velvet program, and the mate-pair reads were analyzed with the SOAPaligner/soap2 tool to identify the relationship between different contigs (17). Remaining gaps were closed by specific PCR and Sanger sequencing, using the ABI 3730 system (Applied Biosystems). Contigs and PCR products were assembled by using the Phred/Phrap/consed software package (Codon-Code Corporation). The annotation was performed by using a RAST server and the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>). Genomic islands (GIs) were analyzed with the IslandViewer software tool (6). The metabolic pathways of aromatic and heterocyclic compounds were examined through KEGG databases (5). Comparative genome analysis was performed with Mauve (2).

The genome of strain S16 consists of a single circular chromosome that is 5,984,790 bp in length, with a GC content of 62.3% and without plasmids. The genome encodes 5,218 putative coding sequences (CDS; 974-bp average length; 85%

coding density), of which 4,058 CDS have functional predictions. It contains 6 ribosomal operons, an extra 5S rRNA, and 70 tRNA loci. Numerous predicted mobile elements were identified in the S16 genome that bring some additional features to the strain. Compared with four other *P. putida* strains, KT2440, W619, F1, and GB-1 (16), strain S16 shares 3,298 CDS with all of them (80% identity on 80% of the protein length). The genome contains genes related to catabolism of aromatic and heterocyclic compounds, such as nicotine, benzoate, and phenylalanine. The enzymes NicA (10) and 6-hydroxy-3-succinoylpyridine hydroxylase (11) in the first two steps of nicotine degradation are located between bp 449,898 and 453,055 in the S16 chromosome. Twenty-nine GIs were identified in the genome. The biggest GI (43,989 bp in length) has a G+C content of 52.4% and 8 transposases. More genes associated with nicotine degradation were predicted and identified in this GI (unpublished data). Further analysis of these genes will provide more useful information to understand the full mechanism of nicotine degradation.

Nucleotide sequence accession number. The genome sequence of *P. putida* S16 has been deposited in the GenBank database under accession number CP002870.

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