Pseudomonas putida strain ND6 is an efficient naphthalene-degrading bacterium. The complete genome of strain ND6 was sequenced and annotated. The genes encoding the enzymes involved in catechol degradation by the ortho-cleavage pathway were found in the chromosomal sequence, which indicated that strain ND6 is able to metabolize naphthalene by the catechol meta- and ortho-cleavage pathways.

Pseudomonas putida strain ND6 is capable of utilizing naphthalene as a sole carbon and energy source for growth. As reported previously, the naphthalene-degrading genes of P. putida ND6 were located on a plasmid of 101,858 bp, pND6-1 (6), encoding the enzymes for the conversion of naphthalene to tricarboxylic acid cycle intermediates through the catechol meta-cleavage pathway. Most of the naphthalene catabolic genes of pND6-1 have 99 to 100% identity to the amino acid sequences of their counterparts found in plasmid pDTG1 (3) and NAH7 (10). Moreover, P. putida ND6 harbors a cryptic plasmid of 117,003 bp, pND6-2, which has been sequenced and annotated (GenBank accession no. CP003589). The plasmid contains 32 coding sequences (CDSs) encoding proteins associated with plasmid conjugative transfer, which can assist the naphthalene catabolic plasmid pND6-1 without any conjugative genes being transferred from P. putida ND6 to Escherichia coli AD256 (unpublished data).

The genome of P. putida ND6 was sequenced with a combined strategy using Roche 454 pyrosequencing (7) and Illumina sequencing by synthesis. The low-quality sequences were trimmed before assembly. The Illumina mate-paired reads (1,356.0 Mbp; 226× coverage) generated by Solexa sequencer were assembled by SOAPdenovo (5). Then, the 454 reads (114.9 Mbp; 18.9× coverage) and the split fragments of contigs generated by SOAPdenovo were used for a hybrid assembly with the Newbler sequence assembler (version 2.6). To finish the genome, conventional Sanger sequencing technologies were used to fill the gaps. Coding sequences were predicted by Glimmer3 (2). Functional assignment and classification were obtained by performing sequence similarity search with BLAST (E-value cutoff, 1E-5) (1) against the eggNOG database (8), the KEGG reference database (4), and the nonredundant GenBank CDS database.

The genome of strain ND6 contains a linear chromosome of 6,085,449 bp with 62% GC content. The chromosome encodes 6,153 putative coding sequences (average length, 891 bp), including 19 rRNA genes and 74 tRNA genes.

Comparison with the complete genome sequences of other Pseudomonas putida strains (i.e., KT2440 [NC_002947.3], W619 [NC_009512.1], F1 [NC_010501.1], GB-1 [NC_010322.1], S16 [NC_015733.1], and BIRD-1 [NC_017530.1]) showed that strain ND6 shares 3,174 (53.16%) homologous proteins with all of them (80% identity on 80% of protein length). Comparative analysis revealed that the chromosome contains genes encoding enzymes involved in the metabolism of aromatic compounds, such as tol-uene, xylene, benzoate, and naphthalene. The genes encoding the complete degradation of catechol by the ortho-cleavage pathway are also located in the chromosome. Catechol 1,2-dioxygenase (CatA), muconate lactonizing enzyme (CatB), and muconolactone isomerase (CatC) catalyze the first three key steps of catechol degradation by the ortho-cleavage pathway (9). Three entire catA genes are distributed in the chromosome (located between bp 5080876 and 5839519). The coding genes of catB and catC are organized as an operon (located between bp 5837087 and 5838534). The potential regulator gene catR was found in the upstream region of the catB operon. These findings suggest that strain ND6 is able to metabolize naphthalene by the catechol meta- and ortho-cleavage pathways.

Nucleotide sequence accession number. The complete genome sequence of Pseudomonas putida strain ND6 has been deposited in NCBI GenBank under the accession number CP003588.