

Complete Genome Sequence of the Type Strain *Cupriavidus necator* N-1^{∇†}

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Here we announce the complete genome sequence of the copper-resistant bacterium *Cupriavidus necator* N-1, the type strain of the genus *Cupriavidus*. The genome consists of two chromosomes and two circular plasmids. Based on genome comparison, the chromosomes of *C. necator* N-1 share a high degree of similarity with the two chromosomal replicons of the bioplastic-producing hydrogen bacterium *Ralstonia eutropha* H16. The two strains differ in their plasmids and the presence of hydrogenase genes, which are absent in strain N-1.

Cupriavidus necator N-1, the type strain of the genus *Cupriavidus*, was isolated from soil and described as a nonobligate predator of soil bacteria and fungi (1, 4). A characteristic of this betaproteobacterium is resistance against (heavy) metals. Analyses of the 16S rRNA gene sequence indicated that the bioplastic-producing “Knallgas” bacterium *Ralstonia eutropha* H16, reclassified as *Cupriavidus necator* H16 (1, 2, 3, 5), is closely related to *C. necator* N-1.

Whole-genome shotgun sequencing of *C. necator* N-1 genome was performed by using a 454 GS-FLX system (Roche 454 Life Science, Mannheim, Germany). Four shotgun and one paired-end pyrosequencing runs resulted in 32-fold coverage. The initial assembly yielded 64 scaffolds containing 1,004 contigs. PCR-based techniques and Sanger sequencing of the products were used to close remaining gaps. The final genome sequence of *C. necator* N-1 (8,480,857 bp) comprises two chromosomes of 3,872,936 (chromosome 1) and 2,684,606 (chromosome 2) bp and two circular plasmids of 1,499,175 (pBB1) and 424,140 (pBB2) bp. Chromosomes 1 and 2 harbor 3,646 and 2,468 predicted protein-encoding genes, respectively. In addition, three and two rRNA operons were located on chromosomes 1 and 2, respectively. The two plasmids contain 1,424 (pBB1) and 409 (pBB2) putative protein-encoding genes. The main set of tRNA genes (57) was located on chromosome 1, whereas seven tRNA genes were found on chromosome 2 and two on each of the plasmids.

The genome of *C. necator* N-1 differs in size and the number of replicons from the genome of *R. eutropha* H16, which consists of two chromosomes (4,052,032 and 2,912,490 bp) and one megaplasmid (452,156 bp) adding up to a total size of 7,416,678 bp (2, 3). Bidirectional BLAST analysis revealed that 81% (2,951) and 67% (1,653) of the putative genes located on chromosome 1 and chromosome 2

of *C. necator* N-1 show orthologous counterparts in the genome of *R. eutropha* H16 (e-value less than 1e-20). The percentage of orthologous genes located on pBB1 and pBB2 was only 10 and 19%, respectively. While *C. necator* N-1 harbors one *cbb* operon coding for enzymes of autotrophic CO₂ fixation, *R. eutropha* H16 hosts two copies of the *cbb* operon. The genome of *C. necator* N-1 shares four operons encoding different formate dehydrogenases (chromosome 1, *fds* and *fdh* operons; chromosome 2, *fdo* and *fdw* operons), but putative genes encoding H₂-oxidizing hydrogenases, which enable strain H16 to grow lithoautotrophically (2), were not detected in *C. necator* N-1. Complete sets of genes required for polyhydroxyalkanoate metabolism, degradation of benzoate, 4-hydroxybenzoate, phenol, and chlorinated aromatic compounds, and the gentisate pathway were identified in the *C. necator* N-1 genome. N-1 was described as a copper-resistant bacterium, leading to the genus designation *Cupriavidus* (1, 5). Putative genes coding for copper resistance proteins (*copABCD*) and a copper efflux P-type ATPase (*copF*) belong to the genomic repertoire of *C. necator* N-1. The biological activity of these genes remains to be investigated.

Nucleotide sequence accession numbers. The complete sequences of the *C. necator* N-1 chromosomes and plasmids have been deposited in GenBank under accession numbers CP002877 (chromosome 1), CP002878 (chromosome 2), CP002879 (pBB1), and CP002880 (pBB2).

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