

Genome Sequences of *Alicyclophilus denitrificans* Strains BC and K601^T∇

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***Alicyclophilus denitrificans* strain BC and *A. denitrificans* strain K601^T degrade cyclic hydrocarbons. These strains have been isolated from a mixture of wastewater treatment plant material and benzene-polluted soil and from a wastewater treatment plant, respectively, suggesting their role in bioremediation of soil and water. Although the strains are phylogenetically closely related, there are some clear physiological differences. The hydrocarbon cyclohexanol, for example, can be degraded by strain K601^T but not by strain BC. Furthermore, both strains can use nitrate and oxygen as an electron acceptor, but only strain BC can use chlorate as electron acceptor. To better understand the nitrate and chlorate reduction mechanisms coupled to the oxidation of cyclic compounds, the genomes of *A. denitrificans* strains BC and K601^T were sequenced. Here, we report the complete genome sequences of *A. denitrificans* strains BC and K601^T.**

Alicyclophilus denitrificans strain BC and the closely related *A. denitrificans* strain K601^T are able to grow with benzene aerobically (6, 8). Furthermore, only strain BC can degrade benzene using chlorate as an electron acceptor (8). During chlorate reduction, molecular oxygen is formed, which might be used by oxygenases to attack benzene (7, 8). Cyclohexanol can be degraded by strain K601^T but not by strain BC. Cyclohexanol degradation does not require oxygenases, as was recently reported (4). As comparative genomics of strains BC and K601^T may give more insight into anoxic and oxic degradation of cyclic hydrocarbons, the genomes of strains BC and K601^T were sequenced.

The genomes of strains BC and K601^T were sequenced and assembled at the DOE Joint Genome Institute, and annotation was performed at Oak Ridge National Laboratory (project 4086861). The genomes were based on data from Illumina (2) and 454 (5) technologies, which provided 135× coverage of the genome of strain BC and 416.3× coverage of the genome of strain K601^T. Gene annotation was conducted using Prodigal (3).

The total size of the strain BC genome is 4,835,713 bp, slightly smaller than the strain K601^T genome (5,070,751 bp). The G+C contents of the genomes are comparable, 67.8% for strain K601^T and 67.9% for strain BC. These values are close to those of 66% and 67.7%, respectively, previously deter-

mined by conventional techniques (6, 8). Both genomes are comprised of a circular chromosome, which has a size of 4,637,013 bp in strain BC and 4,995,263 bp in strain K601^T, and both contain plasmids. Strain BC contains a 78,982-bp plasmid and a 119,718-bp megaplasmid, whereas strain K601^T contains one plasmid of 75,488 bp. In total, the genome of strain BC contained 4,545 candidate genes, whereas the genome of strain K601^T contained 4,698 gene models.

Remarkably, candidate genes encoding chlorate reductase and chlorite dismutase are localized on the megaplasmid of strain BC, which is absent in strain K601^T. This finding explains the predicted horizontal gene transfer of genes involved in chlorate respiration (1). Furthermore, genes coding for a benzene/phenol monooxygenase (BC-BMOa) and a catechol 2,3-dioxygenase (BC-C23O), both postulated to be involved in benzene degradation (8), are localized in one gene cluster in the chromosomes of strain BC and strain K601^T. According to our findings, a gene encoding bifunctional hydratase/alcohol dehydrogenase, which is involved in cyclohexanol degradation, was identified in the genome of strain K601^T but not in the genome of strain BC.

The genomes of strains BC and K601^T contain the blueprints of cyclic hydrocarbon degradation under anoxic and oxic conditions. Interestingly, strain BC can employ aerobic benzene degradation pathways under essentially anaerobic conditions. More detailed genome analysis may lead to the development of methods for application of the strains in *in situ* bioremediation of polluted anaerobic soil sites.

Nucleotide sequence accession numbers. The sequences of the chromosomes and plasmids of *A. denitrificans* strains BC and K601^T have been deposited in GenBank. For strain BC, the accession

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numbers are CP002449 (chromosome), CP002450 (megaplasmid), and CP002451 (plasmid). For strain K601^T, the accession numbers are CP002657 (chromosome) and CP002658 (plasmid).

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REFERENCES

1. **Bender, K. S., M. R. Rice, W. H. Fugate, J. D. Coates, and L. A. Achenbach.** 2004. Metabolic primers for detection of (per)chlorate-reducing bacteria in the environment and phylogenetic analysis of *cld* gene sequences. *Appl. Environ. Microbiol.* **70**:5651–5658.
2. **Bennett, S.** 2004. Solexa Ltd. *Pharmacogenomics* **5**:433–438.
3. **Hyatt, D., et al.** 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**:119.
4. **Jin, J., A. J. J. Straathof, M. W. H. Pinkse, and U. Hanefeld.** 2011. Purification, identification, and cloning of a bifunctional molybdoenzyme with hydratase and alcohol dehydrogenase activity. *Appl. Microbiol. Biotechnol.* doi:10.1007/s00253-010-2996-2.
5. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
6. **Mechichi, T., E. Stackebrandt, and G. Fuchs.** 2003. *Alicyclophilus denitrificans* gen. nov., sp. nov., a cyclohexanol-degrading, nitrate-reducing, β -proteobacterium. *Int. J. Syst. Evol. Microbiol.* **53**:147–152.
7. **van Ginkel, C. G., G. B. Rikken, A. G. M. Kroon, and S. W. M. Kengen.** 1996. Purification and characterization of chlorite dismutase: a novel oxygen-generating enzyme. *Arch. Microbiol.* **166**:321–326.
8. **Weelink, S. A. B., et al.** 2008. Isolation and characterization of *Alicyclophilus denitrificans* strain BC, which grows on benzene with chlorate as the electron acceptor. *Appl. Environ. Microbiol.* **74**:6672–6681.