

Complete Genome Sequence of the Haloaromatic Acid-Degrading Bacterium *Achromobacter xylosoxidans* A8[∇]

Hynek Strnad,[†] Jakub Ridl,[†] Jan Paces, Michal Kolar, Cestmir Vlcek,^{*} and Vaclav Paces^{*}

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, 14220 Prague, Czech Republic

Received 28 October 2010/Accepted 12 November 2010

***Achromobacter xylosoxidans* strain A8 was isolated from soil contaminated with polychlorinated biphenyls. It can use 2-chlorobenzoate and 2,5-dichlorobenzoate as sole sources of carbon and energy. This property makes it a good starting microorganism for further development toward a bioremediation tool. The genome of *A. xylosoxidans* consists of a 7-Mb chromosome and two large plasmids (98 kb and 248 kb). Besides genes for the utilization of xenobiotic organic substrates, it contains genes associated with pathogenesis, toxin production, and resistance. Here, we report the complete genome sequence.**

Achromobacter xylosoxidans A8 was isolated from soil contaminated with polychlorinated biphenyls (15). It is able to use 2-chlorobenzoate and 2,5-dichlorobenzoate as sole sources of carbon and energy (7, 8). Its genome consists of a single chromosome comprising 7,013,095 bp, one circular conjugative plasmid, pA81, of 98,156 bp, and one circular plasmid, pA82, of 247,895 bp. The chromosome has relatively high GC content (66%). The plasmids have GC contents of 62%. Whole-genome shotgun and paired-end sequencing were performed by using 454 technology (13). We produced 1,022,247 shotgun reads, 494,741 short paired-end reads (3 kb), and 623,041 long paired-end reads (8 kb) with an average read length of 319 bases. The total number of sequenced bases is 681,709,459, representing a sequencing depth of 92. Automatic assembly was done using Newbler 2.3 software (Roche) and yielded three scaffolds corresponding to three replicons with 65 gaps. The gaps were closed by local assembly of selected reads. The complete sequence was analyzed with Critica (2), Glimmer (5), and Prodigal (6) for the protein-coding genes, tRNAscan (12, 16) and Aragorn (11) for the tRNA and tmRNA genes, and RNAmmer (10) for the rRNA genes. The functions of the predicted protein-coding genes were annotated by comparing our annotation (based on homologs in the UniRef90 [18], NCBI-NR [3], and KEGG [9] databases) with annotation provided by RAST Annotation Server (1) and IGS Annotation Engine (<http://manatee.sourceforge.net>) (17). The annotation results were verified using Artemis (4) with dicodon usage plots (14).

We found 6,459 open reading frames (ORFs) in the chromosome, 104 ORFs in plasmid pA81, and 252 ORFs in plasmid pA82. There are 3 rRNA operons, 60 tRNA genes for all 20 amino acids and selenocystein, and one tmRNA gene, all of them located on the chromosome. The coding density of the *A. xylosoxidans* genome is 91.3%. Functions were assigned to

5,620 ORFs (82.5%). Seven hundred eighty-three ORFs (11.5%) represent genes with some similarity to hypothetical genes in databases. The remaining 412 ORFs have no homologues in the databases (e-value < 10⁻¹⁰). Based on the GC skew analysis and the orientation of transcription, the origin of replication has been localized to the vicinity of the *dnaA* gene.

There are 59 putative intact or mutated transposase genes and phage-related genes. We found a complete *mocpRABCD* gene cluster and a dioxygenase gene for chlorobenzoate degradation (7), a *hyb* operon for salicylate 5-hydroxylase degradation (8), and about 70 dioxygenase genes, some of which may be associated with the bioremediation ability of this bacterial strain. Eight genes are probably responsible for the resistance to heavy metals (metal efflux P-type ATPase). There are 28 genes associated with pathogenesis and 33 genes associated with toxin production and resistance. About 1,000 genes participate in transport phenomena, and 675 genes have regulatory functions. We identified eight pseudogenes that are truncated or inactivated.

Nucleotide sequence accession numbers. The nucleotide sequences were deposited in GenBank with accession numbers CP002287 (chromosome), CP002288 (plasmid pA81), and CP002289 (plasmid pA82).

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REFERENCES

1. Aziz, R. K., et al. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* **9**:75.
2. Badger, J. H., and G. J. Olsen. 1999. CRITICA: coding region identification tool invoking comparative analysis. *Mol. Biol. Evol.* **16**:512–524.
3. Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and E. W. Sayers. 2010. GenBank. *Nucleic Acids Res.* **38**:46–51.
4. Carver, T., et al. 2008. Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. *Bioinformatics* **24**:2672–2676.
5. Delcher, A. L., D. Harmon, S. Kasif, O. White, and S. L. Salzberg. 1999. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.* **27**:4636–4641.
6. Hyatt, D., et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinform.* **11**:119.
7. Jencova, V., et al. 2004. Chlorocatechol catabolic enzymes from *Achromobacter xylosoxidans* A8. *Int. Biodeterior. Biodegradation* **54**:175–181.
8. Jencova, V., et al. 2008. Nucleotide sequence, organization and character-

* Corresponding author: Mailing address: Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Prague 4, Czech Republic. Phone: (420) 296443541. Fax: (420) 224311019. E-mail for C. Vlcek: vlcek@img.cas.cz. E-mail for V. Paces: vpaces@img.cas.cz.

[†] These authors contributed equally to this work.

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- ization of the (halo)aromatic acid catabolic plasmid pA81 from *Achromobacter xylosoxidans* A8. Res. Microbiol. **159**:118–127.
9. **Kanehisa, M., et al.** 2008. KEGG for linking genomes to life and the environment. Nucleic Acids Res. **36**:480–484.
 10. **Lagesen, K., et al.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. **35**:3100–3108.
 11. **Laslett, D., and B. Canback.** 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. **32**:11–16.
 12. **Lowe, T. M., and S. R. Eddy.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**:955–964.
 13. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature **437**:376–380.
 14. **Paces, J., and V. Paces.** 2002. DicononUse: the programme for dicodon bias visualization in prokaryotes. Folia Biol. (Praha) **48**:246–249.
 15. **Pavlu, L., et al.** 1999. Characterization of chlorobenzoate degraders isolated from polychlorinated biphenyl-contaminated soil and sediment in the Czech Republic. J. Appl. Microbiol. **87**:381–386.
 16. **Schattner, P., A. N. Brooks, and T. M. Lowe.** 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res. **33**:686–689.
 17. **Strnad, H., et al.** 2010. Complete genome sequence of the photosynthetic purple nonsulfur bacterium *Rhodobacter capsulatus* SB 1003. J. Bacteriol. **192**:3545–3546.
 18. **Tatusov, R. L., E. V. Koonin, and D. J. Lipman.** 1997. A genomic perspective on protein families. Science **278**:631–637.