

Draft Genome Sequence of Chromate-Resistant and Biofilm-Producing Strain *Pseudomonas alcaliphila* 34

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We report the draft genome sequence of *Pseudomonas alcaliphila* 34, a Cr(VI)-hyperresistant and biofilm-producing bacterium that might be used for the bioremediation of chromate-polluted soils. The genome sequence might be helpful in exploring the mechanisms involved in chromium resistance and biofilm formation.

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Pseudomonas alcaliphila 34, previously identified as *Pseudomonas mendocina* with the Biolog-id system (Biolog, Hayward, CA) (1), is a Cr(VI)-hyperresistant and biofilm-producing bacterium isolated from a soil artificially polluted with chromate (2). The strain has been thoroughly characterized in terms of hundreds of biochemical attributes and its chromate-reducing capability in the presence of different carbon and energy sources (1), and in terms of its biofilm development capability and activity in the presence of several stressors (3). Therefore, *P. alcaliphila* 34 does indeed have exceptional abilities and it is a prospective candidate for remediation in environments where chromate contamination occurs together with other stressors (1).

Here, we present the draft genome sequence of *P. alcaliphila* 34 and provide information about the genetic bases that establish its high-level chromium tolerance and biofilm formation. Furthermore, its genome sequence might provide insight into the biotechnological exploitation of the organism for the remediation of chromium-contaminated sites (4).

The *P. alcaliphila* 34 genome was sequenced using Illumina HiSeq 2000 technology. A total of 101,323,720 reads (101 bp long) were assembled using the CLC Genomics Workbench assembler (CLC bio, Denmark), resulting in 1,718-fold coverage of a 5.44-Mb genome distributed in 277 unoriented contigs, and with an overall G+C content of 62%. BLAST (5) analysis on the nonredundant (NR) database allowed us to discard contaminant sequences from organisms unrelated to *Pseudomonas*, such as those from plants and insects.

Contigs were ordered using CONTIGuator 2.3 (6) with the *P. mendocina* NK01 genome, which is the closest available as a reference (GenBank accession no. CP002620.1). Fifty contigs, for a total of 5.27 Mb, were aligned with the reference genome, allowing us to define their relative order; sequences not mapped on the reference genome, corresponding to 13 contigs and a total of 144,392 nucleotides (nt), were added later on to the draft genome. Gaps between contigs were identified and closed using PCR followed by Sanger sequencing and, in some cases, primer walking sequencing of the PCR products. The final draft genome of *P. al-*

caliphila 34 consists of eight supercontigs plus 10 single contigs, with a total length of 5,445,828 bp.

Genome annotation was performed with the Rapid Annotations using Subsystems Technology (RAST) pipeline (7), allowing for the identification of 4,983 protein-coding sequences, 61 tRNAs, and 4 copies of the genes for 5S, 16S, and 23S rRNA, as described for other *Pseudomonas* species (8, 9).

Genome analysis indicated that *P. alcaliphila* 34 possesses a putative *chrBACF* operon (10) that might be responsible for the high chromate resistance of the bacterium. Mercuric and arsenic resistance operons and many genes encoding putative multidrug resistance efflux systems were also identified on the genome. Additionally, genes known to be involved in biofilm formation, including the genes implicated in flagellar and type IV pilus biogenesis and motility (11, 12), plus two alginate biosynthesis gene clusters (13, 14), were identified.

A more detailed analysis of this genome and a comparative analysis with other Cr(VI)-resistant and biofilm-producing bacteria will provide further insight into the specific properties related to these processes.

Nucleotide sequence accession numbers. The draft genome sequence of *P. alcaliphila* 34 has been deposited at DDBJ/EMBL/GenBank under the accession no. [ANGB000000000](https://www.ncbi.nlm.nih.gov/nuccore/ANGB000000000). The version described in this paper is the first version, [ANGB010000001](https://www.ncbi.nlm.nih.gov/nuccore/ANGB010000001).

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REFERENCES

- Viti C, Decorosi F, Tatti E, Giovannetti L. 2007. Characterization of chromate-resistant and -reducing bacteria by traditional means and by a high-throughput phenomic technique for bioremediation purposes. *Biotechnol. Prog.* 23:553–559.
- Viti C, Mini A, Ranalli G, Lustrato G, Giovannetti L. 2006. Response of microbial communities to different doses of chromate in soil microcosms. *Appl. Soil Ecol.* 34:125–139.
- Santopolo L, Marchi E, Frediani L, Decorosi F, Viti C, Giovannetti L.

2012. A novel approach combining the Calgary biofilm device and phenotype microarray for the characterization of the chemical sensitivity of bacterial biofilms. *Biofouling* 28:1023–1032.
4. Viti C, Giovannetti L. 2008. Bioremediation of soils polluted with hexavalent chromium using bacteria: a challenge, p 57–76. *In* Singh SN, Tripathi RD (ed), *Environmental bioremediation technologies*. Springer-Verlag, New York, NY.
 5. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402.
 6. Galardini M, Biondi EG, Bazzicalupo M, Mengoni A. 2011. CONTIGuator: a bacterial genome finishing tool for structural insights on draft genomes. *Source Code Biol. Med.* 6:11.
 7. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75.
 8. Coenye T, Vandamme P. 2003. Intragenomic heterogeneity between multiple 16S ribosomal RNA operons in sequenced bacterial genomes. *FEMS Microbiol. Lett.* 228:45–49.
 9. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrook-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim R, Smith K, Spencer D, Wong GK, Wu Z, Paulsen IT, Reizer J, Saier MH, Hancock RE, Lory S, Olson MV. 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* 406:959–964.
 10. Branco R, Chung AP, Johnston T, Gurel V, Morais P, Zhitkovich A. 2008. The chromate-inducible *chrBACF* operon from the transposable element Tn*OtChr* confers resistance to chromium(VI) and superoxide. *J. Bacteriol.* 190:6996–7003.
 11. Barken KB, Pamp SJ, Yang L, Gjermansen M, Bertrand JJ, Klausen M, Givskov M, Whitchurch CB, Engel JN, Tolker-Nielsen T. 2008. Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in *Pseudomonas aeruginosa* biofilms. *Environ. Microbiol.* 10:2331–2343.
 12. Klausen M, Heydorn A, Ragas P, Lambertsen L, Aaes-Jørgensen A, Molin S, Tolker-Nielsen T. Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. 2003. *Mol. Microbiol.* 48:1511–1524.
 13. Davies DG, Chakrabarty AM, Geesey GG. 1993. Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* 59:1181–1186.
 14. Hentzer M, Teitzel GM, Balzer GJ, Heydorn A, Molin S, Givskov M, Parsek MR. 2001. Alginate overproduction affects *Pseudomonas aeruginosa* biofilm structure and function. *J. Bacteriol.* 183:5395–5401.