



Genome Sequence of the Dichloromethane-Degrading Bacterium *Hyphomicrobium* sp. Strain GJ21

Françoise Bringel,^a Christiaan P. Postema,^b Sophie Mangenot,^c Sabrina Bibi-Triki,^a Pauline Chaignaud,^a Muhammad Farhan Ul Haque,^a Christelle Gruffaz,^a Louis Hermon,^a Yousra Louhichi,^a Bruno Maucourt,^a Emilie E. L. Muller,^a Thierry Nadalig,^a Aurélie Lajus,^{d,e,f} Zoé Rouy,^{d,e,f} Claudine Médigue,^{d,e,f} Valérie Barbe,^c Dick B. Janssen,^b Stéphane Vuilleumier^a

Université de Strasbourg, CNRS, GMGM UMR 7156, Department of Microbiology, Genomics and the Environment, Strasbourg, France^a; Biochemical Laboratory, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands^b; Commissariat à l'Énergie Atomique et aux Énergies Alternatives, Institut de Biologie François-Jacob, Laboratoire de Biologie Moléculaire pour l'Étude des Génomes, Évry, France^c; Commissariat à l'Énergie Atomique et aux Énergies Alternatives, Institut de Biologie François-Jacob, Laboratoire d'Analyse Bioinformatique en Génomique et Métabolisme, Évry, France^d; Centre National de la Recherche Scientifique, UMR 8030, Évry, France^e; UEVE, Université d'Évry, Évry, France^f

ABSTRACT The genome sequence of *Hyphomicrobium* sp. strain GJ21, isolated in the Netherlands from samples of environments contaminated with halogenated pollutants and capable of using dichloromethane as its sole carbon and energy source, was determined.

Dichloromethane (DCM) is one of the most used industrial halogenated solvents worldwide, and its toxicity and substantial release into the environment are of serious concern (1, 2). Various methylotrophic bacterial strains capable of growing with DCM as the sole source of carbon and energy have been isolated over the years (3). All such strains for which the dehalogenase has been characterized, including strain GJ21 (4), contain the *dcmA* gene for DCM dehalogenase, an enzyme of the glutathione *S*-transferase family (5, 6). Other DCM-degrading systems, particularly from anaerobic environments, are currently being investigated in detail (7–10), including at the genome level. Whereas natural sources of DCM exist, such as production by microalgae (11), most DCM in the environment is of industrial origin, raising questions about the origin, evolution, and distribution of DCM dehalogenase genes in microorganisms.

Hyphomicrobium sp. strain GJ21, isolated after prolonged adaptation from a mixture of activated sludge from a wastewater treatment plant and soil samples from polluted sites (12), has been extensively used as a model for bioremediation of DCM in bioreactor studies (e.g., see reference 13 and references therein). The genome of *Hyphomicrobium* sp. strain GJ21 was sequenced using Illumina technology. A mate-paired library (7-kb insert size) and a paired-end library (340-bp insert size) were produced and sequenced using HiSeq2000 (2 × 100 nucleotides), yielding ~4 Gb and ~1.85 Gb of useful reads after selection and trimming, respectively. Sequence data were assembled with the Velvet assembler (<https://www.ebi.ac.uk/~zerbino/velvet>). Gap filling was performed using GapCloser (<http://soap.genomics.org.cn/soapdenovo.html>) on scaffolds larger than 2 kb. The final assembly resulted in 1 circular scaffold comprising 4 contigs with a cumulative size of 3.84 Mb and a G+C content of 60.8%. Automatic and manual gene annotations were performed using the MicroScope platform (14). The genome contains a single rRNA operon and encodes 47 tRNAs for all

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Address correspondence to Stéphane Vuilleumier, vuilleumier@unistra.fr.

amino acids. It features genes for enzymes participating in methanol, methylamine, dimethylamine, and trimethylamine oxidation and associated accessory genes; for enzymes involved in formaldehyde oxidation by the tetrahydromethanopterin pathway, enzymes of the tetrahydrofolate-dependent C1 pathway, and corresponding cofactor biosynthesis genes; and for enzymes involved in formate oxidation, as well as genes of the serine and ethylmalonyl-CoA cycles for carbon assimilation (15). The genome also possesses the strongly conserved *dcm* islet of *dcmRABC* genes found in all bacteria with *dcmA*-encoded DCM dehalogenase (3), including *Hyphomicrobium denitrificans* ATCC 51888 of known genome sequence (16). Genes associated with dissimilatory nitrate reduction and complete denitrification to N₂ were also identified, as expected from the known ability of *Hyphomicrobium* strains to grow with DCM with nitrate as the terminal electron acceptor in the absence of oxygen (17).

Several other *Hyphomicrobium* genome sequences of isolated strains (16, 18–21) (<http://www.genoscope.cns.fr/agc/microscope>) or reconstructed from metagenomes (22) are now publicly available. Comparative genomics of the *Hyphomicrobium* genus will support ongoing experimental studies on bacterial adaptation to growth with halogenated methanes for bioremediation applications.

Accession number(s). The *Hyphomicrobium* sp. strain GJ21 genome sequence was deposited in GenBank under the accession number [CDHO00000000](https://doi.org/10.1016/j.chemosphere.2013.07.022). The version described here is the first version, CDHO01000000.

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REFERENCES

1. Shestakova M, Sillanpää M. 2013. Removal of dichloromethane from ground and wastewater: a review. *Chemosphere* 93:1258–1267. <https://doi.org/10.1016/j.chemosphere.2013.07.022>.
2. Schlosser PM, Bale AS, Gibbons CF, Wilkins A, Cooper GS. 2015. Human health effects of dichloromethane: key findings and scientific issues. *Environ Health Perspect* 123:114–119. <https://doi.org/10.1289/ehp.1308030>.
3. Muller EEL, Bringel F, Vuilleumier S. 2011. Dichloromethane-degrading bacteria in the genomic age. *Res Microbiol* 162:869–876. <https://doi.org/10.1016/j.resmic.2011.01.008>.
4. Vuilleumier S, Ivoš N, Dean M, Leisinger T. 2001. Sequence variation in dichloromethane dehalogenases/glutathione S-transferases. *Microbiology* 147:611–619. <https://doi.org/10.1099/00221287-147-3-611>.
5. Vuilleumier S, Pagni M. 2002. The elusive roles of bacterial glutathione S-transferases: new lessons from genomes. *Appl Microbiol Biotechnol* 58:138–146. <https://doi.org/10.1007/s00253-001-0836-0>.
6. Allocati N, Federici L, Masulli M, Di Ilio C. 2009. Glutathione transferases in bacteria. *FEBS J* 276:58–75. <https://doi.org/10.1111/j.1742-4658.2008.06743.x>.
7. Lee M, Wells E, Wong YK, Koenig J, Adrian L, Richnow HH, Manefield M. 2015. Relative contributions of *Dehalobacter* and zerovalent iron in the degradation of chlorinated methanes. *Environ Sci Technol* 49:4481–4489. <https://doi.org/10.1021/es5052364>.
8. Kleindienst S, Higgins SA, Tsementzi D, Konstantinidis KT, Mack EE, Löffler FE. 2016. Draft genome sequence of a strictly anaerobic dichloromethane-degrading bacterium. *Genome Announc* 4(2):e00037–16. <https://doi.org/10.1128/genomeA.00037-16>.
9. Kleindienst S, Higgins SA, Tsementzi D, Chen G, Konstantinidis KT, Mack EE, Löffler FE. 2017. "*Candidatus* Dichloromethanomonas elyunquensis" gen. nov., sp. nov., a dichloromethane-degrading anaerobe of the *Pepetococcaceae* family. *Syst Appl Microbiol* 40:150–159. <https://doi.org/10.1016/j.syapm.2016.12.001>.
10. Trueba-Santiso A, Parladé E, Rosell M, Lliros M, Mortan SH, Martínez-Alonso M, Gaju N, Martín-González L, Vicent T, Marco-Urrea E. 2017. Molecular and carbon isotopic characterization of an anaerobic stable enrichment culture containing *Dehalobacterium* sp. during dichloromethane fermentation. *Sci Total Environ* 581:640–648. <https://doi.org/10.1016/j.scitotenv.2016.12.174>.
11. Paul C, Pohnert G. 2011. Production and role of volatile halogenated compounds from marine algae. *Nat Prod Rep* 28:186–195. <https://doi.org/10.1039/c0np00043d>.
12. Ottengraf SPP, Meesters JJP, Vandenoever AHC, Rozema HR. 1986. Biological elimination of volatile xenobiotic compounds in biofilters. *Bioproc Eng* 1:61–69. <https://doi.org/10.1007/bf00387497>.
13. Diks RMM, Ottengraf SPP, Vrijlnad S. 1994. The existence of a biological equilibrium in a trickling filter for waste-gas purification. *Biotechnol Bioeng* 44:1279–1287. <https://doi.org/10.1002/bit.260441103>.
14. Vallenet D, Calteau A, Cruveiller S, Gachet M, Lajus A, Josso A, Mercier J, Renaux A, Rollin J, Rouy Z, Roche D, Scarpelli C, Médigue C. 2017. MicroScope in 2017: an expanding and evolving integrated resource for community expertise of microbial genomes. *Nucleic Acids Res* 45:D517–D528. <https://doi.org/10.1093/nar/gkw1101>.
15. Ochsner AM, Sonntag F, Buchhaupt M, Schrader J, Vorholt JA. 2015. *Methylobacterium extorquens*: methylotrophy and biotechnological applications. *Appl Microbiol Biotechnol* 99:517–534. <https://doi.org/10.1007/s00253-014-6240-3>.
16. Brown PJB, Kysela DT, Buechlein A, Hemmerich C, Brun YV. 2011. Genome sequences of eight morphologically diverse alphaproteobacteria. *J Bacteriol* 193:4567–4568. <https://doi.org/10.1128/JB.05453-11>.
17. Kohler-Staub D, Frank S, Leisinger T. 1995. Dichloromethane as the sole carbon source for *Hyphomicrobium* sp. strain DM2 under denitrification conditions. *Biodegradation* 6:229–235. <https://doi.org/10.1007/BF00700462>.
18. Vuilleumier S, Nadalig T, Ul Haque MF, Magdelenat G, Lajus A, Roselli S, Muller EEL, Gruffaz C, Barbe V, Médigue C, Bringel F. 2011. Complete genome sequence of the chloromethane-degrading *Hyphomicrobium* sp. strain MC1. *J Bacteriol* 193:5035–5036. <https://doi.org/10.1128/JB.05627-11>.
19. Venkatramanan R, Prakash O, Woyke T, Chain P, Goodwin LA, Watson D, Brooks S, Kostka JE, Green SJ. 2013. Genome sequences for three denitri-

- fyng bacterial strains isolated from a uranium- and nitrate-contaminated subsurface environment. *Genome Announc* 1(4):e00449-13. <https://doi.org/10.1128/genomeA.00449-13>.
20. Martineau C, Villeneuve C, Mauffrey F, Villemur R. 2014. Complete genome sequence of *Hyphomicrobium nitratorans* strain NL23, a denitrifying bacterium isolated from biofilm of a methanol-fed denitrification system treating seawater at the Montreal Biodome. *Genome Announc* 2(1):e01165-13. <https://doi.org/10.1128/genomeA.01165-13>.
21. Martineau C, Mauffrey F, Villemur R. 2015. Comparative analysis of denitrifying activities of *Hyphomicrobium nitratorans*, *Hyphomicrobium denitrificans*, and *Hyphomicrobium zavarzinii*. *Appl Environ Microbiol* 81:5003–5014. <https://doi.org/10.1128/AEM.00848-15>.
22. Fujinawa K, Asai Y, Miyahara M, Kouzuma A, Abe T, Watanabe K. 2016. Genomic features of uncultured methylotrophs in activated-sludge microbiomes grown under different enrichment procedures. *Sci Rep* 6:26650. <https://doi.org/10.1038/srep26650>.