



Draft Genome Sequence of *Methylobacterium radiotolerans*, a DDE-Degrading and Plant Growth-Promoting Strain Isolated from *Cucurbita pepo*

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We announce the draft genome of *Methylobacterium radiotolerans*, a Gram-negative bacterium isolated from *Cucurbita pepo* roots. This strain shows 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (DDE)-degrading potential and plant growth-promoting capacities. Analyses of its 6.8-Mb genome will improve our understanding of DDE-degradation pathways and aid in the deployment of phytoremediation technologies to remediate DDE-contaminated soils.

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DT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] (1) is a recalcitrant bioaccumulative pesticide introduced in 1943 that has been used for agriculture, for gardening, and to control the spread of diseases such as malaria (2). The primary degradation product in soil, 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (DDE), is classified as a persistent organic pollutant (POP) along with DDT (3). These materials are hormone disrupting and can be acutely and chronically toxic to wildlife (4). Endophyte-enhanced phytoremediation using the DDE-accumulating zucchini plant, *Cucurbita pepo* (5), is currently being explored for soil remediation.

Here, we describe a whole-genome shotgun sequence for *Methylobacterium radiotolerans*, a DDE-degrading strain isolated from the roots of *C. pepo* plants that were grown with 100 mg·liter⁻¹ of DDE. The strain was identified by partial 16S rRNA gene sequencing, and the closest related 16S rRNA sequence (99%) was from strain JCM2831 (GenBank accession no. CP001001.1).

An IonTorrent PGM was used to generate a whole-genome shotgun sequence for M. radiotolerans using methods described by Eevers et al. (6).

A total of 379 Mb of data were generated in Torrent Suite version 4.2.1 and assembled into 240 contigs >1,000 bp using SPAdes version 3.1.0 (7, 8) (uniform coverage mode; *k*-mers 21, 33, 55, 77, 99), giving a consensus length of 6,810,393 bp at a 29× coverage (largest contig, 210,399 bp; N_{50} , 57,566 bp). Contigs were ordered with the genome of *Methylobacterium radiotolerans* JCM2831 as a reference in Mauve (9). Annotation was completed using the PGAP (NCBI) pipeline (10). The genome of *M. radiotolerans* consists of a single circular chromosome (71.19% GC content), including 6,373 coding genes that were arranged into 338 pathways with Pathway Tools (11, 12), 1,474 pseudogenes, 13 rRNAs (5S, 16S, 23S), 50 tRNAs, and 1 noncoding RNA (ncRNA).

In laboratory experiments, *Methylobacterium radiotolerans* exhibited increased growth yields when DDE was added to the medium. Analyses of the draft genome revealed the presence of ha-

logenases, dioxygenases, and hydrolases that have been previously reported to be implicated in DDE degradation (13–16). *Methylobacterium radiotolerans* also possesses genes associated with the degradation of toluene, naphthalene, acrylonitrile, phenylacetate, and nitriloacetate, as well as genes for arsenate detoxification. Genes that code for plant growth-promoting capacities, such as 1-aminocyclopropane-1-carboxylate deaminase activity, siderophore production, auxin biosynthesis, and phosphorous solubilization, are present, and these activities were confirmed *in vitro*. Furthermore, the genome harbors genes for nitrogen fixation, glutathione biosynthesis, and the degradation of superoxide radicals. Given its biodegradative and plant growth-promoting abilities, *Methylobacterium radiotolerans* is being studied as an inoculant for the phytoremediation of DDE-contaminated soils.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JXTO00000000. The version described in this paper is version JTXO01000000.

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REFERENCES

- Thomas JE, Ou LT, All-Agely A. 2008. DDE remediation and degradation. Rev Environ Contam Toxicol 194:55–70. http://dx.doi.org/10.1007/ 978-0-387-74816-0_3.
- 2. Turusov V, Rakitsky V, Tomatis L. 2002. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ Health Perspect 110: 125–128.
- White JC. 2010. Inheritance of *p*,*p*'-DDE phytoextraction ability in hybridized *Cucurbita pepo* cultivars. Environ Sci Technol 44:5165–5169. http://dx.doi.org/10.1021/es100706t.
- 4. Colles A, Koppen G. 2007. DDE serumwaarden in landelijke regio's en de Albertkanaalzone boven het Vlaamse referentiegemiddelde: en-

quete naar de oorzaak. De Biomonito, digitale nieuwsbrief van het medisch milieukundig netwerk (VITO). http://www.milieu-en-gezondheid.be /nieuwsbrief/biomonitor%2017/DDE.html.

- 5. White JC, Parrish ZD, Isleyen M, Gent MPN, Iannucci-Berger W, Eitzer BD, Mattina MJI. 2005. Uptake of weathered *p*,*p*'-DDE by plant species effective at accumulating soil elements. Microchem J 81:148–155. http://dx.doi.org/10.1016/j.microc.2005.01.010.
- Eevers N, Van Hamme JD, Bottos EM, Weyens N, Vangronsveld J. 2015. Draft genome sequence of *Enterobacter aerogenes*, a DDE-degrading and plant growth-promoting strain isolated from *Cucurbita pepo*. Genome Announc 3(2):e00317-15. http://dx.doi.org/10.1128/ genomeA.00317-15.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to singlecell sequencing. J Comput Biol 19:455–477. http://dx.doi.org/10.1089/ cmb.2012.0021.
- 8. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. http://dx.doi.org/10.1093/bioinformatics/btt086.
- Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. 2009. Reordering contigs of draft genomes using the Mauve Aligner. Bioinformatics 25:2071–2073. http://dx.doi.org/10.1093/bioinformatics/btp356.
- Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thom-

son N, White O. 2008. Toward an online repository of standard operating procedures (SOPs) for (meta)genomic annotation. OMICS 12:137–141. http://dx.doi.org/10.1089/omi.2008.0017.

- Caspi R, Altman T, Billington R, Dreher K, Foerster H, Fulcher CA, Holland TA, Keseler IM, Kothari A, Kubo A, Krummenacker M, Latendresse M, Mueller LA, Ong Q, Paley S, Subhraveti P, Weaver DS, Weerasinghe D, Zhang P, Karp PD. 2014. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/ genome databases. Nucleic Acids Res 42:D459–D471. http://dx.doi.org/ 10.1093/nar/gkt1103.
- Karp PD, Paley S, Romero P. 2002. The Pathway Tools software. Bioinformatics 18(Suppl 1):S225–S232. http://dx.doi.org/10.1093/ bioinformatics/18.suppl_1.S225.
- Quensen JF, Mueller SA, Jain MK, Tiedje JM. 1998. Reductive dechlorination of DDE to DDMU in marine sediment microcosms. Science 280: 722–724. http://dx.doi.org/10.1126/science.280.5364.722.
- Hay AG, Focht DD. 1998. Cometabolism of 1,1-dichloro-2,2-bis(4chlorophenyl)ethylene by *Pseudomonas acidovorans* M3GY grown on biphenyl. Appl Environ Microbiol 64:2141–2146.
- Nadeau LJ, Menn FM, Breen A, Sayler GS. 1994. Aerobic degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by *Alcaligenes eutrophus* A5. Appl Environ Microbiol 60:51–55.
- Hay AG, Focht DD. 2000. Transformation of 1,1-dichloro-2,2-(4-chlorophenyl)ethane (DDD) by *Ralstonia eutropha* strain A5. FEMS Microbiol Ecol 31:249–253. http://dx.doi.org/10.1111/j.1574 -6941.2000.tb00690.x.