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Genome Sequence of the Verrucomicrobium *Opitutus terrae* PB90-1, an Abundant Inhabitant of Rice Paddy Soil Ecosystems[▽]

Mark W. J. van Passel,[1] Ravi Kant,[2] Airi Palva,[2] Alex Copeland,[3,4] Susan Lucas,[3,5] Alla Lapidus,[3,4]† Tijana Glavina del Rio,[3,4] Sam Pitluck,[3,4] Eugene Goltsman,[3,4] Alicia Clum,[3,4] Hui Sun,[3,4] Jeremy Schmutz,[6] Frank W. Larimer,[7] Miriam L. Land,[7] Loren Hauser,[7] Nikolaos Kyrpides,[3,4] Natalia Mikhailova,[3,4] P. Paul Richardson,[3,4] Peter H. Janssen,[8] Willem M. de Vos,[1,2] and Hauke Smidt[1]*

Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands[1]; Department of Veterinary Biosciences, Faculty of Veterinary Medicine, FIN-00014 University of Helsinki, Helsinki, Finland[2]; DOE Joint Genome Institute, Walnut Creek, California 94598[3]; Lawrence Berkeley National Laboratory, Berkeley, California 94720[4]; Lawrence Livermore National Laboratory, Livermore, California 94550[5]; HudsonAlpha Genome Sequencing Center, Huntsville, Alabama 35806[6]; Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831[7]; and AgResearch Ltd., Grasslands Research Centre, Palmerston North, New Zealand[8]

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Bacteria of the deeply branching phylum *Verrucomicrobia* are rarely cultured yet commonly detected in metagenomic libraries from aquatic, terrestrial, and intestinal environments. We have sequenced the genome of *Opitutus terrae* PB90-1, a fermentative anaerobe within this phylum, isolated from rice paddy soil and capable of propionate production from plant-derived polysaccharides.

The *Verrucomicrobia* comprise an excellent example of a bacterial phylum that is hardly detected by conventional cultivation-based approaches. However, these bacteria are frequently encountered in 16S rRNA gene libraries from a wide variety of ecosystems, including aquatic and terrestrial environments (20), as well as the mammalian gastrointestinal tract (5, 19). During the past 5 years, improved cultivation techniques have resulted in the isolation of a range of members of this phylum (8). This resulted in the observation that different lineages of the *Verrucomicrobia* have a compartmentalized cell organization, similar to that of the *Planctomycetes* (14, 15). More recently, it has been suggested that the *Planctomycetes-Verrucomicrobia-Chlamydiae* superphylum may have contributed significantly to eukaryogenesis (18), an innovation of considerable interest in biological evolution.

The availability of a comprehensive set of verrucomicrobial genomes will allow comparative genomic analyses of members from this phylum, providing relevant information in the fields of taxonomy and evolution. It will expand the phylogenetic coverage for the comparison of the metabolic capacities of aquatic, terrestrial, and intestinal populations of verrucomicrobia and their interactions with their environments. Recently, a species of the *Verrucomicrobia*, *Verrucomicrobium spinosum*, was shown to be amenable to transformation (6), paving the way for the development of genetic tools in other members of this phylum.

Opitutus terrae PB90-1 is an obligatory anaerobic member of the phylum *Verrucomicrobia.* It was isolated from rice paddy soil (4), along with several closely related strains (2, 11). The metabolism of *Opitutus* spp. seems suited for growth on plant-derived (poly)saccharides in this soil and for interaction with methanogens in response to local hydrogen partial pressures (3, 10).

The genome was sequenced using the GS20 454 pyrosequencing platform in combination with Sanger sequencing (7.53× coverage for pyrosequencing, 12.0× coverage for Sanger). A hybrid 454/Sanger assembly was made using the Arachne assembler. Possible misassemblies were corrected, and gaps between contigs were closed by editing in Consed by custom primer walks from subclones or PCR products.

Coding sequences (CDSs) were predicted using Prodigal (9). GenePrimp (17) was used to manually curate start sites and analyze intergenic regions for missed CDSs. Protein descriptions were predicted using Interpro and BlastP. Noncoding genes and miscellaneous features were predicted using tRNAscan-SE (16), RNAMMer (13), Rfam (7), TMHMM (12), and signalP (1).

The genome of *Opitutus terrae* PB90-1 contains a single circular chromosome of 5,957,605 bp. The overall G+C content of the chromosome is 65.3%, less than the 74% predicted by Chin et al. (4). The chromosome contains 4,632 protein-coding genes (CDSs), 65 tRNA genes, and 3 rRNA genes. A putative function could be predicted for 3,102 (67.0%), and signal peptides are predicted in 1,428 (30.8%) protein coding genes.

Nucleotide sequence accession number. The genome sequence of the type strain *Opitutus terrae* PB90-1 is deposited in GenBank under accession number CP001032.

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[*] Corresponding author. Mailing address: Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB, Wageningen, Netherlands. Phone: 31 317 483102. Fax: 31 317 483829. E-mail: Hauke.Smidt@wur.nl.

[†] Present address: Fox Chase Cancer Center, Philadelphia, PA.

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REFERENCES

1. **Bendtsen, J. D., H. Nielsen, G. von Heijne, and S. Brunak.** 2004. Improved prediction of signal peptides: SignalP 3.0. J. Mol. Biol. **340:**783–795.
2. **Chin, K. J., D. Hahn, U. Hengstmann, W. Liesack, and P. H. Janssen.** 1999. Characterization and identification of numerically abundant culturable bacteria from the anoxic bulk soil of rice paddy microcosms. Appl. Environ. Microbiol. **65:**5042–5049.
3. **Chin, K. J., and P. H. Janssen.** 2002. Propionate formation by *Opitutus terrae* in pure culture and in mixed culture with a hydrogenotrophic methanogen and implications for carbon fluxes in anoxic rice paddy soil. Appl. Environ. Microbiol. **68:**2089–2092.
4. **Chin, K. J., W. Liesack, and P. H. Janssen.** 2001. *Opitutus terrae* gen. nov., sp. nov., to accommodate novel strains of the division "Verrucomicrobia" isolated from rice paddy soil. Int. J. Syst. Evol. Microbiol. **51:**1965–1968.
5. **Derrien, M., M. C. Collado, K. Ben-Amor, S. Salminen, and W. M. de Vos.** 2008. The mucin degrader *Akkermansia muciniphila* is an abundant resident of the human intestinal tract. Appl. Environ. Microbiol. **74:**1646–1648.
6. **Domman, D. B., B. T. Steven, and N. L. Ward.** 2010. Random transposon mutagenesis of *Verrucomicrobium spinosum* DSM 4136^T. Arch. Microbiol. [Epub ahead of print.] doi:10.1007/s00203-010-0666-5.
7. **Griffiths-Jones, S., A. Bateman, M. Marshall, A. Khanna, and S. R. Eddy.** 2003. Rfam: an RNA family database. Nucleic Acids Res. **31:**439–441.[Epub ahead of print.
8. **Hedlund, B. P.** 2011. Verrucomicrobiae phyl. nov., p. 795–799. *In* N. R. Krieg, J. T. Staley, D. R. Brown, B. P. Hedlund, B. J. Paster, N. Ward, W. Ludwig, and W. B. Whitman (ed.), Bergey's manual of systematic bacteriology, 2nd ed., vol. 4. Springer Verlag, New York, NY.
9. **Hyatt, D., et al.** 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics **11:**119.
10. **Janssen, P. H.** 1998. Pathway of glucose catabolism by strain VeGlc2, an anaerobe belonging to the *Verrucomicrobiales* lineage of bacterial descent. Appl. Environ. Microbiol. **64:**4830–4833.
11. **Janssen, P. H., A. Schuhmann, E. Morschel, and F. A. Rainey.** 1997. Novel anaerobic ultramicrobacteria belonging to the Verrucomicrobiales lineage of bacterial descent isolated by dilution culture from anoxic rice paddy soil. Appl. Environ. Microbiol. **63:**1382–1388.
12. **Krogh, A., B. Larsson, G. von Heijne, and E. L. Sonnhammer.** 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. **305:**567–580.
13. **Lagesen, K., et al.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. **35:**3100–3108.
14. **Lee, K. C., et al.** 2009. Phylum Verrucomicrobia representatives share a compartmentalized cell plan with members of bacterial phylum Planctomycetes. BMC Microbiol. **9:**5.
15. **Lindsay, M. R., et al.** 2001. Cell compartmentalisation in planctomycetes: novel types of structural organisation for the bacterial cell. Arch. Microbiol. **175:**413–429.
16. **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.
17. **Pati, A., et al.** 2010. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. Nat. Methods **7:**455–457.
18. **Santarella-Mellwig, R., et al.** 2010. The compartmentalized bacteria of the planctomycetes-verrucomicrobia-chlamydiae superphylum have membrane coat-like proteins. PLoS Biol. **8:**e1000281.
19. **van Passel, M. W. J., et al.** 2011. The genome of *Akkermansia muciniphila*, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. PLoS One **6:**e16876.
20. **Wagner, M., and M. Horn.** 2006. The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr. Opin. Biotechnol. **17:**241–249.