

Genome Sequence of *Bacillus pumilus* MTCC B6033

Jacylyn Villanueva,^a Jack Switala,^a Anabella Ivancich,^b Peter C. Loewen^a

Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada^a; CNRS, Unité de Recherche Mixte CNRS/CEA/Université Paris-Sud (UMR 8221), Laboratoire de Bioénergétique, Métalloprotéines et Stress, Centre d'Etudes de Saclay, iBiTec-S, Gif-sur-Yvette, France^b

***Bacillus pumilus* is a Gram-positive, rod-shaped, aerobic bacterium isolated from the soil. *B. pumilus* strain B6033 was originally selected as a biocatalyst for the stereospecific oxidation of β -lactams. Here, we present a 3.8-Mb assembly of its genome, which is the second fully assembled genome of a *B. pumilus* strain.**

Received 27 March 2014 Accepted 3 April 2014 Published 17 April 2014

Citation Villanueva J, Switala J, Ivancich A, Loewen PC. 2014. Genome sequence of *Bacillus pumilus* MTCC B6033. *Genome Announc.* 2(2):e00327-14. doi:10.1128/genomeA.00327-14.

Copyright © 2014 Villanueva et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Peter C. Loewen, peter.loewen@umanitoba.ca.

Bacillus pumilus is a soil bacterium with cellular features similar to those of other members of the *Bacillus* genus. Various attributes of the species have been exploited industrially, and *B. pumilus* strain B6033, originally isolated in India, was selected in a screen for a biocatalyst to effect the stereospecific oxidation of β -lactams to their (R)-sulfoxide derivatives (1). Subsequently, the enzyme responsible for the oxidation was isolated, and based on its dual capability to react as a catalase (H_2O_2 disproportionation) and a peroxidase (oxidation of a typical substrate), it was concluded to be a catalase-peroxidase (KatG) but with physical properties somewhat different from those of all other characterized KatGs (1). The putative existence of such an unusual KatG was of interest because it had the potential to shed light on the evolution and *in vivo* role of an extensively studied class of relatively new enzymes, but one which still presented many questions (2). KatGs are best known for their role in the activation of isoniazid as an antituberculosis drug, wherein mutations in the *katG* gene give rise to isoniazid resistance in *Mycobacterium tuberculosis* (3). KatGs are phylogenetically and structurally linked to the peroxidase family (4, 5), but their catalytic activity is predominant by several orders of magnitude. A side-by-side comparison has revealed a family with remarkably similar properties, making the enzyme from *B. pumilus* an apparent outlier and therefore interesting in its own right (6). In order to produce the large quantities of protein needed for a complete characterization, we wanted to clone the putative *katG* gene, and therefore, we set out to determine the sequence of the genome.

The genome of *B. pumilus* B6033 was sequenced in two stages. The first stage employed data generated using an Illumina MiSeq platform, which was assembled into 14 contigs using a combination of MIRA Assembler version 3.9.3 (7), Velvet version 1.2.08 (8), the MUMmer version 3.23 (9) package, and some Sanger sequencing. The second stage to complete the genome utilized a Pacific Biosciences data set generated by Genome Québec, which was assembled using the PacBio SMRT Analysis pipeline version 2.0.1, with 172 \times coverage to give a single contiguous genome sequence. The 14 contigs from the Illumina data were aligned for confirmation. The sequence was annotated by the National Center

for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline.

The genome sequence of *B. pumilus* MTCC B6033 consists of 3,763,493 bases, with a G+C content of 41.4%. There are 3,659 putative coding sequences, 81 tRNA genes, and 6 rRNA clusters. A comparison of the genome with the only other completed *B. pumilus* genome, that of strain SAFR-032 (accession no. NC_009848.1) (10), using Mauve 2.3.1 (11) revealed 92% identity, with rearrangements of only two small sections. Relevant to the initial purpose of the work, a catalase-peroxidase gene was not found. However, two functional catalase genes were found, one for a typical clade 1 monofunctional heme catalase and the second for a manganese catalase, in addition to a cryptic gene for a monofunctional catalase.

Nucleotide sequence accession number. The genome sequence of *B. pumilus* MTCC B6033 was deposited with NCBI GenBank under the accession no. [CP007436.1](https://www.ncbi.nlm.nih.gov/nuccore/CP007436.1).

ACKNOWLEDGMENTS

This work was supported by a grant (DG9600) from the Natural Sciences and Engineering Research Council (NSERC) of Canada and by the Canadian Research Chairs Program (CRC), both to P.C.L.

We acknowledge the support of Genome Québec and Genome Canada for funding to the Innovation Centre where the PacBio sequencing was performed. We also acknowledge support for the Manitoba next-generation sequencing platform provided by the Manitoba Institute of Child Health, the CancerCare Manitoba Foundation, the Canadian Foundation for Innovation, Province of Manitoba, University of Manitoba Faculty of Medicine, Manitoba Health Research Council, and Manitoba Institute of Cell Biology. We thank R. S. Jolly and the Microbial Type Culture Collection (IMTECH, Chandigarh, India) for providing us with the *B. pumilus* MTCC B6033 strain.

REFERENCES

1. Sangar S, Pal M, Moon LS, Jolly RS. 2012. A catalase-peroxidase for oxidation of β -lactams to their (R)-sulfoxides. *Bioresour. Technol.* 115: 102–110. <http://dx.doi.org/10.1016/j.biortech.2011.09.045>.
2. Njuma OJ, Ndontsa EN, Goodwin DC. 2014. Catalase in peroxidase clothing: interdependent cooperation of two cofactors in the catalytic versatility of KatG. *Arch. Biochem. Biophys.* 544:27–39. <http://dx.doi.org/10.1016/j.abb.2013.11.007>.

3. Heym B, Alzari PM, Honoré N, Cole ST. 1995. Missense mutations in the catalase-peroxidase gene, *katG*, are associated with isoniazid resistance in *Mycobacterium tuberculosis*. *Mol. Microbiol.* 15:235–245. <http://dx.doi.org/10.1111/j.1365-2958.1995.tb02238.x>.
4. Klotz MG, Loewen PC. 2003. The molecular evolution of catalatic hydroperoxidases: evidence for multiple lateral transfer of genes between prokaryota and from bacteria into eukaryota. *Mol. Biol. Evol.* 20:1098–1112. <http://dx.doi.org/10.1093/molbev/msg129>.
5. Zamocky M, Furtmüller PG, Obinger C. 2008. Evolution of catalases from bacteria to humans. *Antioxid. Redox Signal.* 10:1527–1548. <http://dx.doi.org/10.1089/ars.2008.2046>.
6. Singh R, Wiseman B, Deemagarn T, Jha V, Switala J, Loewen PC. 2008. Comparative study of catalase-peroxidases (KatGs). *Arch. Biochem. Biophys.* 471:207–214. <http://dx.doi.org/10.1016/j.abb.2007.12.008>.
7. Chevreux B, Pfisterer T, Drescher B, Driesel AJ, Müller WE, Wetter T, Suhai S. 2004. Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. *Genome Res.* 14:1147–1159. <http://dx.doi.org/10.1101/gr.1917404>.
8. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
9. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. *Genome Biol.* 5:R12. <http://dx.doi.org/10.1186/gb-2004-5-6-p12>.
10. Tirumalai MR, Rastogi R, Zamani N, O'Bryant Williams E, Allen S, Diouf F, Kwende S, Weinstock GM, Venkateswaran KJ, Fox GE. 2013. Candidate genes that may be responsible for the unusual resistances exhibited by *Bacillus pumilus* SAFR-032 spores. *PLoS One* 8:e66012. <http://dx.doi.org/10.1371/journal.pone.0066012>.
11. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5:e11147. <http://dx.doi.org/10.1371/journal.pone.0011147>.