

# Draft Genome Sequence of *Ralstonia* sp. MD27, a Poly(3-Hydroxybutyrate)-Degrading Bacterium, Isolated from Compost

Morgan Zhu,<sup>a</sup> Lucy M. McCully,<sup>b</sup> Mark W. Silby,<sup>b</sup> Tamunonengiyeofori I. Charles-Ogan,<sup>c</sup> Jean Huang,<sup>a</sup> Christopher J. Brigham<sup>c</sup>

Franklin W. Olin College of Engineering, Needham, Massachusetts, USA<sup>a</sup>; Department of Biology<sup>b</sup> and Department of Bioengineering,<sup>c</sup> University of Massachusetts Dartmouth, North Dartmouth, Massachusetts, USA

***Ralstonia* sp. strain MD27, a novel biopolymer-degrading betaproteobacterium, was isolated from compost samples. This organism has been shown to utilize the biopolymer poly(3-hydroxybutyrate) [P(3HB)] as a carbon source for growth. We report the draft genome sequence of MD27 with an estimated total sequence length of 5.9 Mb.**

Received 24 August 2015 Accepted 25 August 2015 Published 8 October 2015

**Citation** Zhu M, McCully LM, Silby MW, Charles-Ogan TI, Huang J, Brigham CJ. 2015. Draft genome sequence of *Ralstonia* sp. MD27, a poly(3-hydroxybutyrate)-degrading bacterium, isolated from compost. *Genome Announc* 3(5):e01170-15. doi:10.1128/genomeA.01170-15.

**Copyright** © 2015 Zhu 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 Jean Huang, [jean.huang@olin.edu](mailto:jean.huang@olin.edu), or Christopher J. Brigham, [cbrigham@umassd.edu](mailto:cbrigham@umassd.edu).

*Ralstonia* species are a diverse group that includes plant and human pathogens and other soil-dwelling organisms, such as *Ralstonia eutropha* (now known as *Cupriavidus necator*) (1), *Ralstonia solanacearum* (2), and *Ralstonia pickettii* (3, 4). *Ralstonia* and related species like *Cupriavidus necator* synthesize and mobilize poly(3-hydroxybutyrate) [P(3HB)] and other polyhydroxyalkanoate (PHA) biopolymers as part of their natural metabolism (5, 6). PHA has attracted much recent interest as a potential biobased, biodegradable alternative to petroleum-based plastics (7, 8).

*Ralstonia* sp. strain MD27 was isolated from compost from the greenhouse at Wellesley College (Wellesley, MA, USA) using media containing P(3HB) as the sole carbon source. The bacterium grows using P(3HB) with a doubling time of 3.3 h. Genomic DNA of *Ralstonia* sp. MD27 was extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). Libraries were then generated using the Nextera XT sequencing kit (Illumina, San Diego, CA, USA) and sequenced by an Illumina MiSeq at Tufts University Genomics Core Facility. We obtained 2,298,090 2 × 250-bp reads (185-fold coverage), which were assembled into 72 contigs (from 611 to 600,706 bp) using CLC Genomics Workbench version 7.5.

The draft genome sequence of MD27 comprises 5,917,320 bp (63.6% G+C content) and was annotated using the NCBI PGAP pipeline. We predicted 5,382 protein coding sequences, 47 tRNA sequences, and three copies each of 16S, 23S, and 5S rRNA genes. The 16S rDNA sequence of MD27 was found to be most closely related to that of *Ralstonia pickettii* strain 12J (99% identity based on nucleotide BLAST).

Two PHA synthase (*phaC*) genes were predicted (contigs 4 and 6), both of which appear to code for the conserved lipase box necessary for activity (9). We also detected two putative β-ketothiolase (*phaA*) genes (contigs 6 and 12), and two putative acetoacetyl-CoA reductase (*phaB*) genes (contigs 4 and 6). Indeed, the *phaCAB* sequences in contig 6 appear to form a gene cluster similar to a PHA biosynthesis operon found in *C. necator* H16 (10, 11) and *R. pickettii* strain 12D. A putative regulatory gene (*phaR*)

has been identified in contig 6. These findings suggest that MD27 can synthesize intracellular polyhydroxyalkanoates.

Four putative PHA depolymerase (*phaZ*) genes were identified in the MD27 genome: three intracellular depolymerase genes (contigs 2, 6, and 22) and an extracellular P(3HB) depolymerase gene (contig 34) that were predicted, based on a BLAST search with the Depolymerase Engineering Database (DED, <http://www.ded.uni-stuttgart.de>) (12), to be an extracellular short-chain-length PHA depolymerase with a type I catalytic domain, as previously described (13). Experimentally validated PHA depolymerases that also fall into this category include the PhaZ from *Ralstonia pickettii* (14). *Ralstonia* sp. MD27 expresses a P(3HB) depolymerase that is capable of breaking down highly crystalline P(3HB) and is unlike the well-studied model organism of PHA homeostasis, *C. necator* strain H16 (10, 11), implying that *Ralstonia* sp. MD27 could be utilized in composting items made of P(3HB).

**Nucleotide sequence accession numbers.** This whole-genome shotgun sequencing project has been deposited in DDBJ/EMBL/GenBank under accession number [LFZM000000000](https://www.ncbi.nlm.nih.gov/nuccore/LFZM000000000). The version described in this paper is the first version, LFZM01000000.

## ACKNOWLEDGMENTS

This research was supported by seed funding from the Office of the Provost at the University of Massachusetts Dartmouth. We thank the staff at the Wellesley College greenhouse for allowing M. Zhu to procure a compost sample and the spring 2014 Microbial Diversity course at Olin College for discussions and feedback in development of this work.

## REFERENCES

- Riedel SL, Lu J, Stahl U, Brigham CJ. 2014. Lipid and fatty acid metabolism in *Ralstonia eutropha*: relevance for the biotechnological production of value-added products. *Appl Microbiol Biotechnol* 98:1469–1483. <http://dx.doi.org/10.1007/s00253-013-5430-8>.
- Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y. 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* Davis 1969) comb. nov. *Microbiol Immunol* 39:897–904.

3. Hundt K, Wagner M, Becher D, Hammer E, Schauer F. 1998. Effect of selected environmental factors on degradation and mineralization of biaryl compounds by the bacterium *Ralstonia pickettii* in soil and compost. *Chemosphere* 36:2321–2335. [http://dx.doi.org/10.1016/S0045-6535\(97\)10201-6](http://dx.doi.org/10.1016/S0045-6535(97)10201-6).
4. Centers for Disease Control and Prevention (CDC). 1998. Nosocomial *Ralstonia pickettii* colonization associated with intrinsically contaminated saline solution—Los Angeles, California, 1998. *Morb Mortal Wkly Rep* 47:285–286.
5. Riedel SL, Lu J, Stahl U, Brigham CJ. 2014. Lipid and fatty acid metabolism in *Ralstonia eutropha*: relevance for the biotechnological production of value-added products. *Appl Microbiol Biotechnol* 98:1469–1483. <http://dx.doi.org/10.1007/s00253-013-5430-8>.
6. Jendrossek D. 2009. Polyhydroxyalkanoate granules are complex subcellular organelles (carbonosomes). *J Bacteriol* 191:3195–3202. <http://dx.doi.org/10.1128/JB.01723-08>.
7. Brigham CJ, Sinskey AJ. 2012. Applications of polyhydroxyalkanoates in the medical industry. *Int J Biotechnol Well Ind* 1:53–60. <http://dx.doi.org/10.6000/1927-3037.2012.01.01.03>.
8. Brigham CJ, Sinskey AJ. 2012. Polyhydroxyalkanoate production enzymes: a survey and biological perspective. *J Siberian Fed Univ* 3:220–242.
9. Rehm BH. 2003. Polyester synthases: natural catalysts for plastics. *Biochem J* 376:15–33. <http://dx.doi.org/10.1042/BJ20031254>.
10. Peoples OP, Sinskey AJ. 1989. Poly-beta-hydroxybutyrate (PHB) biosynthesis in *Alcaligenes eutrophus* H16: identification and characterization of the PHB polymerase gene (phbC). *J Biol Chem* 264:15298–15303.
11. Peoples OP, Sinskey AJ. 1989. Poly-beta-hydroxybutyrate biosynthesis in *Alcaligenes eutrophus* H16: characterization of the genes encoding beta-ketothiolase and acetoacetyl-CoA reductase. *J Biol Chem* 264:15293–15297.
12. Knoll M, Hamm TM, Wagner F, Martinez V, Pleiss J. 2009. The PHA depolymerase engineering database: a systematic analysis tool for the diverse family of polyhydroxyalkanoate (PHA) depolymerases. *BMC Bioinformatics* 10:89. <http://dx.doi.org/10.1186/1471-2105-10-89>.
13. Behrends A, Klingbeil B, Jendrossek D. 1996. Poly(3-hydroxybutyrate) depolymerases bind to their substrate by a C-terminal located substrate binding site. *FEMS Microbiol Lett* 143:191–194. <http://dx.doi.org/10.1111/j.1574-6968.1996.tb08479.x>.
14. Hiraishi T, Hirahara Y, Doi Y, Maeda M, Taguchi S. 2006. Effects of mutations in the substrate-binding domain of poly[(R)-3-hydroxybutyrate] (PHB) depolymerase from *Ralstonia pickettii* T1 on PHB degradation. *Appl Environ Microbiol* 72:7331–7338. <http://dx.doi.org/10.1128/AEM.01187-06>.