



## Draft Genome Sequence of *Lysinibacillus fusiformis* Strain SW-B9, a Novel Strain for Biotransformation of Isoeugenol to Vanillin

## Liqing Zhao, a,b Guanhui Bao,c Beibei Geng,a Jiangning Song,d,e Yin Lic

Department of Food Science and Engineering, College of Chemistry and Chemical Engineering, Shenzhen University, Shenzhen, Guangdong, China<sup>a</sup>; School of Bioscience and Bioengineering, South China University of Technology, Guangzhou, Guangdong, China<sup>b</sup>; CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China<sup>c</sup>; National Engineering Laboratory for Industrial Enzymes and Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China<sup>d</sup>; Department of Biochemistry and Molecular Biology, Faculty of Medicine, Monash University, Melbourne, Australia<sup>e</sup>

L.Z. and G.B. contributed equally to this work.

Lysinibacillus fusiformis SW-B9 was the first reported strain in *L. fusiformis* showing effective biotransformation of isoeugenol to vanillin. Here, we report the annotated genome of strain SW-B9, which has special pathways for producing vanillin. The genome will provide a genetic basis for better understanding the physiology of this species.

Received 26 February 2015 Accepted 3 March 2015 Published 16 April 2015

Citation Zhao L, Bao G, Geng B, Song J, Li Y. 2015. Draft genome sequence of *Lysinibacillus fusiformis* strain SW-B9, a novel strain for biotransformation of isoeugenol to vanillin. Genome Announc 3(2):e00289-15. doi:10.1128/genomeA.00289-15.

Copyright © 2015 Zhao et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license. Address correspondence to Liqing Zhao, snowyzlj@163.com.

Lysinibacillus fusiformis, which prior to 2007 was known as Bacillus fusiformis, is a Gram-positive nonmotile bacterium of the genus Lysinibacillus (1). The majority of studies on L. fusiformis are related to its pathogenicity (2, 3), and few researchers have focused on its industrial and agricultural roles. We previously isolated L. fusiformis SW-B9 from soil, which can produce high amounts of vanillin from isoeugenol (4). Vanillin is widely used in the beverage, food, pharmaceutical, and medical industries (5). Several strains have been shown to be able to convert eugenol or isoeugenol to vanillin (5, 6). However, for L. fusiformis, only the SW-B9 strain was reported for its biotransformation of isoeugenol to vanillin (4). Therefore, the complete sequencing of strain SW-B9 will not only enrich the genome sequence database of L. fusiformis but also further our understanding of the genetic, phylogenetic, and physiological properties of this strain.

The genomic DNA of strain SW-B9 was sequenced using the MiSeq system. The whole-genome shotgun run yielded 2,430,573 paired-end reads, accounting for 1,220,147,646 bases in total. *De novo* assembly was performed using SPAdes (7), resulting in an assembly of 22 contigs of >1,000 bp. The total size of the assembly was 4.7 Mbp, with an  $N_{50}$  of 997.350 kbp and a G+C content of 37%. The annotation of the genome was accomplished with the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) (8). A total of 4,400 coding DNA sequences, 70 tRNAs, and 28 rRNAs were predicted. Out of all the genes, 1,721 coding sequences (CDSs) were assigned to 178 KEGG pathways.

A comparative genome analysis was used to measure the similarities between strain SW-B9 and other strains using the reciprocal smallest distance (RSD) (9). A total of 4,040 CDSs, accounting for 92% of the coding genes in the SW-B9 strain, are shared with *L. fusiformis* RB-21 (GenBank accession no. JPEF00000000.1). These results indicate that most of the coding sequences in the SW-B9 strain are highly conserved within other

L. fusiformis strains, although there is no vanillin biotransformation in these strains. Monooxygenases were considered to be the enzymes that convert isoeugenol to vanillin (10). To find the isoeugenol monooxygenase gene (iem), we used the iem genes reported in Pseudomonas putida IE27 (11), Pseudomonas nitroreducens Jin1 (12), Neofusicoccum parvum isolate UCR-NP2 (13), Verticillium alfalfae VaMs.102, and Colletotrichum fioriniae PJ7 (14), to blast against all proteins in the SW-B9 strain. The results show that there is no similar gene in strain SW-B9. However, in the genome of strain SW-B9, we found 15 candidate monooxygenases, enabling an exploration of the mechanism of vanillin conversion in this strain. Therefore, we speculate that the pathway to produce vanillin in SW-B9 is special, and further genetic research will be worthwhile.

The genome sequence of strain SW-B9 serves as a useful resource for further investigation of the molecular basis of its potential in the biotransformation from isoeugenol to vanillin. Systematic annotations of the genome will reveal physiological differences among the various *L. fusiformis* strains.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. JRBA00000000. The version described in this article is the first version, JRBA01000000.

## **ACKNOWLEDGMENTS**

This work was supported by the Shenzhen Dedicated Funding of Strategic Emerging Industry Development Program (grant JCYJ20140418091413576). J.S. and Y.L. are recipients of the Hundreds Talents Program of the Chinese Academy of Sciences (CAS).

## **REFERENCES**

1. Ahmed I, Yokota A, Yamazoe A, Fujiwara T. 2007. Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* 

- to Lysinibacillus fusiformis comb. nov. and Bacillus sphaericus to Lysinibacillus sphaericus comb. nov. Int J Syst Evol Microbiol 57:1117–1125. http://dx.doi.org/10.1099/ijs.0.63867-0.
- Smith EC. 1933. Inoculation experiments with *Bacillus fusiformis* isolated from tropical ulcer with observations on the bacillus. J Hyg (Lond) 33: 95–102.3.
- Wang J, Fan Y, Yao Z. 2010. Isolation of a *Lysinibacillus fusiformis* strain with tetrodotoxin-producing ability from puffer fish *Fugu obscurus* and the characterization of this strain. Toxicon 56:640–643. http://dx.doi.org/ 10.1016/j.toxicon.2010.05.011.
- Zhao LQ, Sun ZH, Zheng P, Zhu LL. 2005. Biotransformation of isoeugenol to vanillin by a novel strain of *Bacillus fusiformis*. Biotechnol Lett 27:1505–1509. http://dx.doi.org/10.1007/s10529-005-1466-x.
- Priefert H, Rabenhorst J, Steinbüchel A. 2001. Biotechnological production of vanillin. Appl Microbiol Biotechnol 56:296–314. http://dx.doi.org/10.1007/s002530100687.
- Shimoni E, Ravid U, Shoham Y. 2000. Isolation of a *Bacillus* sp. capable of transforming isoeugenol to vanillin. J Biotechnol 78:1–9. http:// dx.doi.org/10.1016/S0168-1656(99)00199-6.
- 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 single-cell sequencing. J Comput Biol 19:455–477. http://dx.doi.org/10.1089/cmb.2012.0021.
- 8. Pruitt KD, Tatusova T, Klimke W, Maglott DR. 2009. NCBI reference

- sequences: current status, policy and new initiatives. Nucleic Acids Res 37:D32–D36. http://dx.doi.org/10.1093/nar/gkn721.
- 9. DeLuca TF, Cui J, Jung JY, St Gabriel KC, Wall DP. 2012. Roundup 2.0: enabling comparative genomics for over 1800 genomes. Bioinformatics 28:715–716. http://dx.doi.org/10.1093/bioinformatics/bts006.
- Hua D, Ma C, Lin S, Song L, Deng Z, Maomy Z, Zhang Z, Yu B, Xu P. 2007. Biotransformation of isoeugenol to vanillin by a newly isolated *Bacillus pumilus* strain: identification of major metabolites. J Biotechnol 130: 463–470. http://dx.doi.org/10.1016/j.jbiotec.2007.05.003.
- Yamada M, Okada Y, Yoshida T, Nagasawa T. 2007. Purification, characterization and gene cloning of isoeugenol-degrading enzyme from *Pseudomonas putida* IE27. Arch Microbiol 187:511–517. http://dx.doi.org/10.1007/s00203-007-0218-9.
- Ryu JY, Seo J, Unno T, Ahn JH, Yan T, Sadowsky MJ, Hur HG. 2010. Isoeugenol monooxygenase and its putative regulatory gene are located in the eugenol metabolic gene cluster in *Pseudomonas nitroreducens* Jin1. Arch Microbiol 192:201–209. http://dx.doi.org/10.1007/s00203-010-0547-y.
- 13. Blanco-Ulate B, Rolshausen P, Cantu D. 2013. Draft genome sequence of *Neofusicoccum parvum* isolate UCR-NP2, a fungal vascular pathogen associated with grapevine cankers. Genome Announc 1(3):e00339-13. http://dx.doi.org/10.1128/genomeA.00339-13.
- 14. Baroncelli R, Sreenivasaprasad S, Sukno SA, Thon MR, Holub E. 2014. Draft genome sequence of *Colletotrichum acutatum sensu lato (Colletotrichum fioriniae*). Genome Announc 2(2):e00112-14. http://dx.doi.org/10.1128/genomeA.00112-14.