Draft Genome Sequence of *Sporolactobacillus inulinus* Strain CASD, an Efficient D-Lactic Acid-Producing Bacterium with High-Concentration Lactate Tolerance Capability

Bo Yu,^{1*} Fei Su,² Limin Wang,¹ Ke Xu,² Bo Zhao,¹ and Ping Xu^{2*}

*Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China,¹ and
State Key Laboratory of Microbial Metabolism and School of Life Sciences and Biotechnology,
Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China²*

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***Sporolactobacillus inulinus* CASD is an efficient D-lactic acid producer with high optical purity. Here we report for the first time the draft genome sequence of *S. inulinus* (2,930,096 bp). The large number of annotated two-component system genes makes it possible to explore the mechanism of extraordinary lactate tolerance of *S. inulinus* CASD.**

Lactic acid, the most important hydroxycarboxylic acid, is regarded as a feedstock for the green chemistry of the future. Besides its versatile applications in the food, pharmaceutical, textile, and leather industries, lactic acid is also a building block for biodegradable plastic, mainly poly(L-lactic acid), which has gained substantial attention in the new wave of industrial biotechnology (7, 4). The development of a stereocomplex of poly(L-lactic acid) and poly(D-lactic acid) is considered an advance in poly(lactic acid) modification, in which the melting point of this stereocomplex could reach the same temperature as that of other petroleum-based polymers (3). Therefore, the potential utilization of D-lactic acid has attracted increased attention to its production by using sustainable bioresource materials (8, 12).

Sporolactobacillus is a genus of anaerobic endospore-forming, Gram-positive, motile, rod-shaped lactic acid bacteria. *Sporolactobacillus inulinus*, the type species of the genus, was originally named *Sporolactobacillus* (subgen. *Lactobacillus*) *inulinus* (5). Because of its high-optical-purity D-lactic acid-producing capability, *Sporolactobacillus inulinus* has been suggested to be a better producer of D-lactic acid (2, 12). *Sporolactobacillus inulinus* CASD, isolated by our group (10), can produce high concentrations of D-lactic acid (207 g/liter) with very high optical purity at 99.3%, which is the highest production titer reported to date (9). The strain has been deposited in the China General Microbiological Culture Collection Center (no. 2185).

Here we report for the first time the draft genome sequence of *Sporolactobacillus inulinus*, obtained using the Illumina GA system with a paired-end library. The reads were assembled with the VELVET software program (11). The draft genome sequence of *S. inulinus* CASD was annotated with the Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (6). In addition, the contigs were searched

against the KEGG, UniProt, and Clusters of Orthologous Groups (COG) databases to annotate the gene descriptions. The G+C mole percent measurements were calculated using the genome sequence.

The draft genome includes 2,930,096 bases with a G+C content of 44.97%, consisting of 674 large contigs (>300 bp in size) and comprised of 3,476 predicted coding sequences (CDSs). According to KEGG pathway analysis, most genes encoding proteins for glycolysis, amino acids, nucleotides, lipids, cofactor/vitamin metabolism, and importantly D-lactic acid formation were successfully annotated. With many fructose- and mannose-metabolizing genes annotated, *S. inulinus* CASD is predicted to be capable of metabolizing fructose and mannose. Furthermore, two-component systems serve as a basic stimulus-response coupling mechanism to allow organisms to sense and respond to changes in many different environmental conditions, including temperature, osmolarity, chemoattractants, and pH pressure (8, 1). A large number of two-component system genes were annotated from the draft genome sequence, which gives a good chance to explore the mechanism of the extraordinary high-concentration lactate tolerance of *S. inulinus* CASD.

Nucleotide sequence accession number. The draft genome sequence of *Sporolactobacillus inulinus* CASD is available in GenBank under accession number AFVQ00000000. The version described in this article is the first version.

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REFERENCES

1. Buckler, D. R., G. S. Anand, and A. M. Stock. 2000. Response-regulator phosphorylation and activation: a two-way street? *Trends Microbiol.* 8:153–156.
2. Fukushima, K., K. Sogo, S. Miura, and Y. Kimura. 2004. Production of D-lactic acid by bacterial fermentation of rice starch. *Macromol. Biosci.* 4:1021–1027.
3. Fukushima, K., Y. H. Chang, and Y. Kimura. 2007. Enhanced stereocomplex formation of poly(L-lactic acid) and poly(D-lactic acid) in the presence of stereoblock poly(lactic acid). *Macromol. Biosci.* 7:829–835.
4. Gao, C., C. Q. Ma, and P. Xu. 6 August 2011. Biotechnological routes based

* Corresponding author. Mailing address for P. Xu: State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China. Phone: 86-21-34206647. Fax: 86-21-34206723. E-mail: pingxu@sjtu.edu.cn. Mailing address for B. Yu: Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China. Phone and fax: 86-10-62555203. E-mail: yub@im.ac.cn.

- on lactic acid production from biomass. *Biotechnol. Adv.* doi:10.1016/j.biotechadv.2011.07.022.
5. **Kitahara, K., and J. Suzuki.** 1963. *Sporolactobacillus* nov. subgen. *J. Gen. Appl. Microbiol.* **9**:59–71.
 6. **Pruitt, K. D., T. Tatusova, W. Klimke, and D. R. Maglott.** 2009. NCBI Reference Sequences: current status, policy and new initiatives. *Nucleic Acids Res.* **37**:D32–D36.
 7. **Qin, J. Y., et al.** 2009. Non-sterilized fermentative production of polymer-grade L-lactic acid by a newly isolated thermophilic strain *Bacillus* sp. 2-6. *PLoS One* **4**:e4359.
 8. **Stock, A. M., V. L. Robinson, and P. N. Goudreau.** 2000. Two-component signal transduction. *Annu. Rev. Biochem.* **69**:183–215.
 9. **Wang, L. M., et al.** 2011. Highly efficient production of D-lactate by *Sporolactobacillus* sp. CASD with simultaneous enzymatic hydrolysis of peanut meal. *Appl. Microbiol. Biotechnol.* **89**:1009–1017.
 10. **Xu, P., et al.** 2007. Method and a *Sporolactobacillus inulinus* strain CASD CGMCC no. 2185 for the production of D-lactic acid. Chinese patent ZL 200710176056.2.
 11. **Zerbino, D. R., and E. Birney.** 2008. VELVET: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* **18**:821–829.
 12. **Zhao, B., et al.** 2010. Kinetics of D-lactic acid production by *Sporolactobacillus* sp. strain CASD using repeated batch fermentation. *Bioresour. Technol.* **101**:6499–6505.