

Genome Sequence of *Enterobacter cloacae* subsp. *dissolvens* SDM, an Efficient Biomass-Utilizing Producer of Platform Chemical 2,3-Butanediol

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***Enterobacter cloacae* subsp. *dissolvens* SDM has an extraordinary characteristic of biomass utilization for 2,3-butanediol production. Here we present a 4.9-Mb assembly of its genome. The key genes for regulation and metabolism of 2,3-butanediol production were annotated, which could provide further insights into the molecular mechanism of high-yield production of 2,3-butanediol.**

The energy crisis of recent years shows the shortage of nonrenewable resources such as crude oil (4, 13). With growing demand for nonrenewable resources and their shortage, biological production of fuels and chemicals from renewable resources has gained great attention (13). 2,3-Butanediol (2,3-BD) is a useful chemical that can be produced from renewable resources (3, 15). It is an important platform compound, which could be used to produce a series of useful products, such as methyl ethyl ketone and 1,3-butadiene (2, 4). Microbial production of 2,3-BD has a long history (over 100 years) (4). Various bacteria were used to produce 2,3-BD (6, 7, 9, 15, 16). However, there has been only one report related to 2,3-BD production by an *Enterobacter* strain, and the production yield was very low (14). The *Enterobacter cloacae* subsp. *dissolvens* SDM (CGMCC 4230) strain isolated from soil samples is a potential industrial candidate for 2,3-BD production (6), which could produce more than 100 g liter⁻¹ 2,3-BD from glucose. Moreover, strain SDM has an extraordinary characteristic of biomass utilization for 2,3-BD production. This strain could efficiently use pentose sugars, xylose, and arabinose from lignocellulose and other cheap biomass, such as cassava, one of the most efficient crops in terms of carbohydrate production (11) for 2,3-BD production (unpublished results).

Here we determined a draft genome sequence of strain SDM. The genome sequence was determined by 454 genome sequencer (454 GS FLX) (10). The genome sequence contains 286,872 reads with an average length of 356 bp at more than 20-fold coverage with the G+C content of 55.1%. The reads were assembled using the Newbler Assembler (454 Life Science) into 168 large contigs (>500 bp) with a length of 4,923,170 bp. The genome sequence was annotated by the RAST server (1). tRNAs were predicted by tRNAscan-SE v.1.23 (8), and rRNAs were found with the RNAmmer 1.2 (5).

The genome sequence of strain SDM contains 4,539 protein-coding sequences (CDSs). Three rRNAs and 53 tRNAs were identified. Seventeen CDSs for the metabolism of pentose sugars from biomass are annotated, which are related to the complete pentose metabolic pathway, including 2 subpathways of phosphoketolase and transketolase/transaldolase. This metabolism is important for pentose utilization in the industrial application. In addition, there are 712 CDSs for utilization of other carbohydrates, indicating that strain SDM may have a wide substrate spectrum. The sequence contains the complete operon (2 CDSs) and key coding

genes (2 CDSs) for 2,3-BD metabolism, which could provide further insights into efficient biomass utilization for the production of 2,3-BD. The genome sequence also contains a series of membrane transport systems, including 56 CDSs involved in ATP-binding cassette (ABC) transporters. The ABC transporters play important roles in not only accumulating compatible solutes and substrates but also excreting unwanted products (12). It may be an important reason for the high-yield production of 2,3-BD.

Nucleotide sequence accession numbers. The whole-genome shotgun sequence has been deposited at DDBJ/EMBL/GenBank under accession number [AGSY000000000](https://www.ncbi.nlm.nih.gov/nuccore/AGSY000000000). The version described in this paper is the first version, with accession number [AGSY010000000](https://www.ncbi.nlm.nih.gov/nuccore/AGSY010000000).

ACKNOWLEDGMENTS

We thank Huajun Zheng and his colleagues for genome sequencing performed at the Chinese National Human Genome Center in Shanghai, People's Republic of China.

This work was supported by grants from the National Natural Science Foundation of China (31000014, 31070062, and 30821005) and the Chinese National Program for High Technology Research and Development (2011AA02A207).

REFERENCES

1. Aziz RK, et al. 2008. The RAST server: rapid annotations using subsystem technology. *BMC Genomics* 9:75.
2. Celińska E, Grajek W. 2009. Biotechnological production of 2,3-butanediol—current state and prospects. *Biotechnol. Adv.* 27:715–725.
3. Ji XJ, et al. 2009. Enhanced 2,3-butanediol production by *Klebsiella oxytoca* using a two-stage agitation speed control strategy. *Bioresour. Technol.* 100:3410–3414.
4. Ji XJ, Huang H, Ouyang PK. 2011. Microbial 2,3-butanediol production: a state-of-the-art review. *Biotechnol. Adv.* 29:351–364.

Received 20 November 2011 Accepted 29 November 2011

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doi:10.1128/JB.06495-11

5. Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of rRNA genes. *Nucleic Acids Res.* **35**:3100–3108.
6. Li L, et al. 27 August 2011. Biocatalytic production of (2S,3S)-2,3-butanediol from diacetyl using whole cells of engineered *Escherichia coli*. *Bioresour. Technol.* doi:10.1016/j.biortech.2011.08.097. [Epub ahead of print].
7. Liu Z, et al. 2011. Production of (2S,3S)-2,3-butanediol and (3S)-acetoin from glucose using resting cells of *Klebsiella pneumoniae* and *Bacillus subtilis*. *Bioresour. Technol.* **102**:10741–10744.
8. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25**:955–964.
9. Ma C, et al. 2009. Enhanced 2,3-butanediol production by *Klebsiella pneumoniae* SDM. *Appl. Microbiol. Biotechnol.* **82**:49–57.
10. Margulies M, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
11. Peters D. 2007. Raw materials. *Adv. Biochem. Eng. Biotechnol.* **105**:1–30.
12. Poolman B. 2002. Transporters and their roles in LAB cell physiology. *Antonie Van Leeuwenhoek* **82**:147–164.
13. Ragauskas AJ, et al. 2006. The path forward for biofuels and biomaterials. *Science* **311**:484–498.
14. Saha BC, Bothast RJ. 1999. Production of 2,3-butanediol by newly isolated *Enterobacter cloacae*. *Appl. Microbiol. Biotechnol.* **52**:321–326.
15. Wang A, et al. 2010. Production of 2,3-butanediol from corn cob molasses, a waste by-product in xylitol production. *Appl. Microbiol. Biotechnol.* **87**:965–970.
16. Zhang L, et al. 2010. Microbial production of 2,3-butanediol by a surfactant (serrawettin)-deficient mutant of *Serratia marcescens* H30. *J. Ind. Microbiol. Biotechnol.* **37**:857–862.