



Complete Genome Sequence of *Actinobacillus succinogenes* GXAS137, a Highly Efficient Producer of Succinic Acid

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ABSTRACT The bacterium *Actinobacillus succinogenes* GXAS137, an efficient producer of succinic acid, was isolated from bovine rumen in Nanning, Guangxi Province, China. Here, we present the 2.3-Mb genome assembly of this strain, which consists of 2,314,479 bp (G+C content of 44.89%) with a circular chromosome, 2,235 DNA coding sequences, 57 tRNAs, and 15 rRNAs.

Succinic acid (SA) is an important C4-building chemical platform for many applications, including food, agriculture, and pharmaceutical (1, 2) production. Commercial SA is mainly produced through petrochemical processes, which bring environmental pollution and other concerns related to sustainable development (3, 4). As an alternative, SA can be manufactured using bio-based feedstock through microbial fermentation (5).

SA production has been accomplished by different microorganisms, including *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Basfia succiniciproducens*, *Mannheimia succiniciproducens* (6), *Saccharomyces cerevisiae* (7), and *Escherichia coli* (8). Although these organisms have achieved a competitive performance, *A. succinogenes*, a capnophilic anaerobic Gram-negative bacterium, is promising because of its ability to form SA naturally at appreciable yields and productivities from a broad range of carbon sources (9–11).

Recent study in our laboratory has isolated an efficient SA-producing strain, *A. succinogenes* GXAS137 (China Center for Type Culture Collection accession no. CCTCC M 2011399), which was originally isolated from bovine rumen in China. The strain can produce up to 95 g/liter of SA with different substrates, such as glucose, cane molasses, duckweed powder, cassava powder, and crude glycerol (12–14). To generate genomic insights into its SA production and relative gene regulation, we performed the whole-genome sequencing of *A. succinogenes* GXAS137.

The genome of *A. succinogenes* GXAS137 was sequenced at Beijing Novogene Bioinformatics Technology Co., Ltd., with massive parallel sequencing using Illumina technology. Two DNA libraries were constructed: a paired-end library with an insert size of 350 bp and a mate-pair library with an insert size of 6 kb. The 350-bp library was sequenced with an Illumina MiSeq and HiSeq 2500 platforms using a paired-end 300-bp strategy. A total of 700 Mb of filtered paired-end reads were obtained with SOAPdenovo software to reach a depth genome coverage of 200-fold (15). Gaps were closed by PCR and subsequent Sanger sequencing. Gene prediction was performed with GeneMarkS. The filtered reads were assembled with SOAPdenovo to generate scaffolds. Genome annotation was predicted with the NCBI Prokaryotic Genome Automatic Annotation Pipeline, and additional software was used to predict the other

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elements in the genome. tRNAs were predicted with tRNAscan-SE, rRNAs were predicted with rRNAmmer, and small RNAs (sRNAs) were predicted by a BLAST search against the Rfam database. PFAST was used for prophage prediction, and CRISPRFinder was used to identify clustered regularly interspaced short palindromic repeats (16).

The genome of *A. succinogenes* GXAS137 was characterized by a circular chromosome of 2,312,173 bp with a 44.89% G+C content without plasmids. The chromosome contains approximately 2,454 predicted genes, 2,235 protein-coding genes, 139 pseudogenes, 57 tRNAs, 15 rRNAs, and 8 sRNAs.

So far, only one complete sequencing genome of the *A. succinogenes* 130Z has been published and analyzed in detail (17). The availability of the complete genome sequence of strain GXAS137 not only will contribute to enriching the genome database but will also give us the opportunity to investigate further the genes related to the biosynthesis of SA.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. [NHRD000000000](https://www.ncbi.nlm.nih.gov/nuclink/NHRD000000000). The version described in this paper is the first version, NHRD01000000.

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REFERENCES

1. Pleissner D, Venus J. 2016. Utilization of protein-rich residues in biotechnological processes. *Appl Microbiol Biotechnol* 100:2133–2140. <https://doi.org/10.1007/s00253-015-7278-6>.
2. Guarnieri MT, Chou YC, Salvachúa D, Mohagheghi A, John PCS, Peterson DJ, Bomble YJ, Beckham GT. 2017. Metabolic engineering of *Actinobacillus succinogenes* provides insights into succinic acid biosynthesis. *Appl Environ Microbiol*. <https://doi.org/10.1128/AEM.00996-17>.
3. Ferone M, Raganati F, Olivieri G, Salatino P, Marzocchella A. 2017. Biosuccinic acid from lignocellulosic-based hexoses and pentoses by *Actinobacillus succinogenes*: characterization of the conversion process. *Appl Biochem Biotechnol* 183:1465–1477. <https://doi.org/10.1007/s12010-017-2514-4>.
4. Shen N, Zhang H, Qin Y, Wang Q, Zhu J, Li Y, Jiang MG, Huang RB. 2018. Efficient production of succinic acid from duckweed (*Landoltia punctata*) hydrolysate by *Actinobacillus succinogenes* GXAS137. *Bioresource Technol* 250:35–42. <https://doi.org/10.1016/j.biortech.2017.09.208>.
5. Ahn JH, Jang YS, Lee SY. 2016. Production of succinic acid by metabolically engineered microorganisms. *Curr Opin Biotech* 42:54–66. <https://doi.org/10.1016/j.copbio.2016.02.034>.
6. Pateraki C, Patsalou M, Vlysidis A, Kopsahelis N, Webb C, Koutinas AA, Koutinas M. 2016. *Actinobacillus succinogenes*: advances on succinic acid production and prospects for development of integrated biorefineries. *Bioch Eng J* 112:285–303. <https://doi.org/10.1016/j.bej.2016.04.005>.
7. Ito Y, Hirasawa T, Shimizu H. 2014. Metabolic engineering of *Saccharomyces cerevisiae* to improve succinic acid production based on metabolic profiling. *Biosci Biotech Bioch* 78:151–159. <https://doi.org/10.1080/09168451.2014.877816>.
8. Olajuyin AM, Yang M, Liu Y, Mu TZ, Tian JN, Adaramoye OA, Xing JM. 2016. Efficient production of succinic acid from *Palmaria palmata* hydrolysate by metabolically engineered *Escherichia coli*. *Bioresource Technol* 214:653–659. <https://doi.org/10.1016/j.biortech.2016.04.117>.
9. Dessie W, Zhang W, Xin F, Dong W, Zhang M, Ma J, Jiang M. 2018. Succinic acid production from fruit and vegetable wastes hydrolyzed by on-site enzyme mixtures through solid state fermentation. *Bioresource Technol* 247:1177–1180. <https://doi.org/10.1016/j.biortech.2017.08.171>.
10. Jiang M, Ma J, Wu M, Liu RM, Liang LY, Xin FX, Zhang WM, Jia HH, Dong WL. 2017. Progress of succinic acid production from renewable resources: metabolic and fermentative strategies. *Bioresource Technol* 245:1710–1717. <https://doi.org/10.1016/j.biortech.2017.05.209>.
11. Gunnarsson IB, Karakashev D, Angelidaki I. 2014. Succinic acid production by fermentation of *Jerusalem artichoke* tuber hydrolysate with *Actinobacillus succinogenes* 130Z. *Ind Crop Prod* 62:125–129. <https://doi.org/10.1016/j.indcrop.2014.08.023>.
12. Shen N, Wang Q, Qin Y, Zhu J, Zhu QX, Mi HZ, Wei YT, Huang RB. 2014. Optimization of succinic acid production from cane molasses by *Actinobacillus succinogenes* GXAS137 using response surface methodology (RSM). *Food Sci Biotechnol* 23:1911–1919. <https://doi.org/10.1007/s10068-014-0261-7>.
13. Shen N, Wang Q, Zhu J, Qin Y, Liao S, Li Y, Zhu Q, Jin Y, Du L, Huang R. 2016. Succinic acid production from duckweed (*Landoltia punctata*) hydrolysate by batch fermentation of *Actinobacillus succinogenes* GXAS137. *Bioresource Technol* 211:307–312. <https://doi.org/10.1016/j.biortech.2016.03.036>.
14. Shen N, Liao SM, Wang Q, Qin Y, Zhu QX, Zhu J, Li Y, Huang RB. 2016. Economical succinic acid production from sugarcane juice by *Actinobacillus succinogenes* supplemented with corn steep liquor and peanut meal as nitrogen sources. *Sugar Tech* 18:292–298. <https://doi.org/10.1007/s12355-015-0401-2>.
15. Mu TZ, Zhou JZ, Yang MH, Xing JM. 2016. Complete genome sequence of *Thialkalicoccus versutus* D301 isolated from soda lake in northern China, a typical strain with great ability to oxidize sulfide. *J Biotechnol* 227:21–22. <https://doi.org/10.1016/j.jbiotec.2016.04.019>.
16. Fang L-C, Chen Y-F, Zhou Y-L, Wang D-S, Sun L-N, Tang X-Y, Hua R-M. 2016. Complete genome sequence of a novel chlorpyrifos degrading bacterium, *Cupriavidus nantongensis* X1. *J Biotechnol* 227:1–2. <https://doi.org/10.1016/j.jbiotec.2016.04.012>.
17. McKinlay JB, Laivenieks M, Schindler BD, McKinlay AA, Siddaramappa S, Challacombe JF, Lowry SR, Clum A, Lapidus AL, Burkhart KB, Harkins V, Vieille C. 2010. A genomic perspective on the potential of *Actinobacillus succinogenes* for industrial succinate production. *BMC Genomics* 11:680. <https://doi.org/10.1186/1471-2164-11-680>.