2011

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JOURNAL OF BACTERIOLOGY, Aug. 2011, p. 4019–4020 Vol. 193, No. 15
0021-9193/11/$12.00 doi:10.1128/JB.05180-11

Complete Genome Sequence of *Lactobacillus buchneri* NRRL B-30929, a Novel Strain from a Commercial Ethanol Plant▽

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Received 28 April 2011/Accepted 10 May 2011

Lactobacillus buchneri **strain NRRL B-30929 was a contaminant obtained from a commercial ethanol fermentation. This facultative anaerobe is unique because of its rapid growth on xylose and simultaneous fermentation of xylose and glucose. The strain utilizes a broad range of carbohydrate substrates and possesses a high tolerance to ethanol and other stresses, making it an attractive candidate for bioconversion of biomass substrates to various bioproducts. The genome sequence of NRRL B-30929 will provide insight into the unique properties of this lactic acid bacterium.**

Lactobacillus buchneri is a heterofermentative, facultative anaerobe that belongs to the lactic acid bacteria. Strains of *L. buchneri* have been described as having diverse activities, ranging from prevention of silage spoilage by yeasts and molds (1, 2, 10) to histamine production in Swiss cheese (9). Several strains have been reported to metabolize lactate to produce 1,2-propanediol (5, 7) and to produce 1,3-propanediol from glycerol (8, 11, 12). A sauerkraut isolate of *L. buchneri* was found to produce an antibacterial peptide that inhibited the growth of selected Gram-positive bacteria (13–15). Interestingly, several isolates of *L. buchneri* are capable of producing ferulate esterases, which break down the cross-links between lignin and hemicellulose (6).

The strain NRRL B-30929 was originally isolated from an ethanol production plant and can tolerate high ethanol concentrations (4). *L. buchneri* NRRL B-30929 is unique in its rapid growth on xylose and ability to simultaneously ferment glucose and xylose. In addition, the strain can utilize a broad spectrum of monosaccharides, disaccharides, and oligosaccharide substrates, and it can tolerate inhibitors present in lignocellulosic hydrolysates (3).

The general methods of genomic DNA preparation, library construction, and sequencing can be found on the Joint Genome Institute (JGI) website (http://www.jgi.doe.gov/sequencing/protocols/index.html). A whole-genome shotgun strategy using Roche 454 Titanium pyrosequencing was performed, and DNA sequences were processed and assembled by the JGI. NRRL B-30929 has one circular chromosome of 2,506,301 bp, with a G+C content of 44.4%, and three plasmids, pLBU01 (52,697 bp with 38.1% G+C), pLBU02 (18,513 bp with 40.4% G+C), and pLBU03 (10,798 bp with 37.6% G+C).

The NRRL B-30929 genome contains a total of 2,541 genes with 2,461 predicted coding sequences (CDSs) and 80 genes for RNAs, including 63 tRNA and 15 rRNA genes (https://merced.jgi-psf.org/cgi-bin/er/main.cgi). There are total of 1,976 CDSs (77.76%) with predicted functions and 485 CDSs (19.09%) without predicted functions. A total of 1,965 genes can be associated with clusters of orthologous genes (COGs) functions belonging to 1,174 COGs. In summary, there are 204 genes for amino acid transport and metabolism, 180 genes for replication, recombination, and repair, 171 genes for carbohydrate transport and metabolism, 161 genes for signal transduction mechanisms and transcription, 144 genes for translation, ribosomal structure, and biogenesis, 124 genes involved in cell wall/membrane/envelope biogenesis, 99 genes for inorganic ion transport and metabolism, and 93 genes for energy production and conversion.

Generation of the genome sequence for NRRL B-30929 will foster engineering of carbohydrate metabolism and modification of end product profiles for biofuels and other platform chemicals. Moreover, the finished genome sequence will enable whole-genome expression studies to better understand the stress response systems present in this ethanol-tolerant microbe.

Nucleotide sequence accession numbers. The complete genome sequence of *Lactobacillus buchneri* NRRL B-30929 is available in GenBank under the accession number CP002652. The accession numbers for the plasmids pLBU01, pLBU02, and pLBU03 are CP002653, CP002654, and CP002655, respectively.

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▽ Published ahead of print on 27 May 2011.

We thank and acknowledge all of the JGI personnel who participated in sequencing, assembly, and automated annotation of the *L. buchneri* genome project. We thank Joseph Rich for reading the manuscript and Jacqueline Zane and Lucy Joseph for their excellent technical support.

This work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under contract no. DE-AC02-05CH11231.

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REFERENCES

1. **Kleinschmit, D. H., R. J. Schmidt, and L. Kung, Jr.** 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. J. Dairy Sci. **88:**2130–2139.
2. **Kung, L., Jr., and N. K. Ranjit.** 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. J. Dairy Sci. **84:**1149–1155.
3. **Liu, S., et al.** 2009. Conversion of biomass hydrolysates and other substrates to ethanol and other chemicals by *Lactobacillus buchneri*. Lett. Appl. Microbiol. **48:**337–342.
4. **Liu, S., K. A. Skinner-Nemec, and T. D. Leathers.** 2008. *Lactobacillus buchneri* strain NRRL B-30929 converts a concentrated mixture of xylose and glucose into ethanol and other products. J. Ind. Microbiol. Biotechnol. **35:** 75–81.
5. **Nishino, N., M. Yoshida, H. Shiota, and E. Sakaguchi.** 2003. Accumulation of 1,2-propanediol and enhancement of aerobic stability in whole crop maize silage inoculated with *Lactobacillus buchneri*. J. Appl. Microbiol. **94:**800–807.
6. **Nsereko, V. L., et al.** 2006. The American Dairy Science Association annual meeting abstract.
7. **Oude Elferink, S. J., et al.** 2001. Anaerobic conversion of lactic acid to acetic acid and 1, 2-propanediol by *Lactobacillus buchneri*. Appl. Environ. Microbiol. **67:**125–132.
8. **Schutz, H., and F. Radler.** 1984. Anaerobic reduction of glycerol to propanediol-1.3 by *Lactobacillus brevis* and *Lactobacillus buchneri*. Syst. Appl. Microbiol. **5:**169–178.
9. **Sumner, S. S., M. W. Speckhard, E. B. Somers, and S. L. Taylor.** 1985. Isolation of histamine-producing *Lactobacillus buchneri* from Swiss cheese implicated in a food poisoning outbreak. Appl. Environ. Microbiol. **50:**1094–1096.
10. **Taylor, C. C., N. J. Ranjit, J. A. Mills, J. M. Neylon, and L. Kung, Jr.** 2002. The effect of treating whole-plant barley with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for dairy cows. J. Dairy Sci. **85:**1793–1800.
11. **Veiga-da-Cunha, M., and M. A. Foster.** 1992. 1,3-Propanediol:NAD+ oxidoreductases of *Lactobacillus brevis* and *Lactobacillus buchneri*. Appl. Environ. Microbiol. **58:**2005–2010.
12. **Veiga da Cunha, M., and M. A. Foster.** 1992. Sugar-glycerol cofermentations in lactobacilli: the fate of lactate. J. Bacteriol. **174:**1013–1019.
13. **Yildirim, M.** 2001. Characterization of buchnericin LB produced by *Lactobacillus buchneri* LB. Turk. J. Biol. **25:**73–82.
14. **Yildirim, M.** 2001. Purification of buchnericin LB produced by *Lactobacillus buchneri* LB. Turk. J. Biol. **25:**59–65.
15. **Yildirim, Z., Y. K. Avsar, and M. Yildirim.** 2002. Factors affecting the adsorption of buchnericin LB, a bacteriocin produced by *Lactobacillus buchneri*. Microbiol. Res. **157:**103–107.