

## Genome Sequence of the Naturally Plasmid-Free Lactobacillus plantarum Strain NC8 (CCUG 61730)

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*Lactobacillus plantarum* is a highly versatile lactic acid bacterium found in various ecological niches, such as fermented vegetable, meat, and dairy products and the gastrointestinal tract. We sequenced the genome of *L. plantarum* NC8, a naturally plasmid-free strain, which has been used as a model strain in many laboratories worldwide.

Lactic acid bacteria (LAB) are important in several fermentation processes and as commensals/symbionts in the gastrointestinal (GI) tract (3, 20). One of the most versatile LAB species is *Lactobacillus plantarum* (16). Strains of *L. plantarum* can colonize the GI tract (1, 10) as well as dominate many fermentations of food and feed products (7, 11). The versatility and metabolic capacity make *L. plantarum* interesting for industrial applications, and some strains are marketed as starter cultures or probiotics (7, 9, 22). *L. plantarum* WCFS1 was the first *Lactobacillus* strain to be genome sequenced (6) and was recently resequenced and reannotated (15). Presently, three additional *L. plantarum* genomes are publically available (17, 21, 23).

*L. plantarum* strain NC8 (CCUG 61730) was isolated in the 1980s from grass silage and identified as naturally plasmid free, an unusual property among *L. plantarum* strains. NC8 has been used as a model strain in the development of genetic tools, such as conjugation (14), transformation (2), and expression vectors (18, 19), in lactobacilli. It has also been used in studies of bacteriocin production (8), general fermentations (4), and metabolic engineering (13). Here, we report the genome sequence of *L. plantarum* NC8.

Whole-genome sequencing was performed (GATC, Konstanz, Germany) with a combined strategy using 454 single-end sequencing (500,381 reads totaling ~220 Mb; ~69-fold coverage) on a GS FLX platform and Illumina paired-end sequencing with an insert length of 300 bp. The initial assembly of 454 reads was with Newbler, and contigs were further assembled using pairedend reads together with mapping to the reference genome of L. plantarum WCFS1 (6, 15). The assembled draft genome, consisting of 10 contigs with a total length of 3,207,224 bp and 44.5% GC content, has 2,868 predicted protein-coding sequences (CDS), 64 pseudogenes, 5 rRNA operons, 70 tRNA genes, and no plasmids. Automatic annotation was done with the Institute for Genome Sciences (IGS) pipeline (http://ae.igs.umaryland.edu/cgi/index .cgi) and curated using the highly homologous curated genome of L. plantarum WCFS1 (15). New CDS were manually annotated using NCBI BLAST and InterProScan (12).

Comparison with published genomes of *L. plantarum* strains WCFS1 (6, 15), ATCC 14917 (GenBank accession number NZ\_ACGZ0000000.2), JDM1 (23), and ST-III (21) shows that all of the genomes are highly colinear with the exception of the type and location of prophages; some prophages of NC8 are novel,

while others are present in strain WCFS1 or JDM1. Most of the orthologous genes of strains NC8, WCFS1, and ATCC 14917 have >98% nucleotide sequence identity. Exceptions include gene clusters for extracellular polysaccharide (EPS)/capsular polysaccharide (CPS) biosynthesis which are variable in gene content and nucleotide sequence identity in all *L. plantarum* genomes (17); strain NC8 shares some of these EPS/CPS genes with other strains but also has a novel EPS/CPS cluster of 10 genes. An NC8 cluster of 12 genes encodes a type III restriction-modification system not present in the other genomes. Only strains NC8 and ATCC 14917 encode a tannase (tannin acyl hydrolase), which is produced by some microorganisms to assist in the degradation of plant-derived tannins into gallic acid (5).

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number AGRI00000000. The version described in this paper is the first version, AGRI01000000.

## ACKNOWLEDGMENTS

We thank the Institute for Genome Sciences (IGS), University of Maryland, School of Medicine, for automatic genome annotation.

This project was jointly funded by The Research Council of Norway (project 192558), Nofima AS, the Foundation for Levy on Foods (Norway), the Netherlands Bioinformatics Centre (NBIC), Nijmegen, and Top Institute Food and Nutrition, Wageningen, The Netherlands.

## REFERENCES

- 1. Ahrné S, et al. 1998. The normal *Lactobacillus* flora of healthy human oral and rectal mucosa. J. Appl. Microbiol. 85:88–94.
- 2. Aukrust T, Blom H. 1992. Transformation of *Lactobacillus* strains used in meat and vegetable fermentations. Food Res. Int. 25:253–261.
- 3. Axelsson L, Ahrné S. 2000. Lactic acid bacteria, p 365–386. *In* Priest FG, Goodfellow M (ed), Applied microbial systematics. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Baardseth P, et al. 2006. Lactic acid fermentation reduces acrylamide formation and other Maillard reactions in French fries. J. Food Sci. 71: C28–C33.

Received 14 February 2012 Accepted 20 February 2012 Address correspondence to Lars Axelsson, lars.axelsson@nofima.no. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.00141-12

- 5. Belur PD, Mugeraya G. 2011. Microbial production of tannase: state of the art. Res. J. Microbiol. 6:25–40.
- Kleerebezem M, et al. 2003. Complete genome sequence of *Lactobacillus plantarum* WCFS1. Proc. Natl. Acad. Sci. U. S. A. 100:1990–1995.
- 7. Luxananil P, et al. 2009. Monitoring *Lactobacillus plantarum* BCC 9546 starter culture during fermentation of Nham, a traditional Thai pork sausage. Int. J. Food Microbiol. **129**:312–315.
- Maldonado A, Ruiz-Barba JL, Jiménez-Díaz R. 2003. Purification and genetic characterization of plantaricin NC8, a novel coculture-inducible two-peptide bacteriocin from *Lactobacillus plantarum* NC8. Appl. Environ. Microbiol. 69:383–389.
- 9. Molin G. 2003. The role of *Lactobacillus plantarum* in foods and in human health, p 305–342. *In* Farnworth E (ed), Handbook of fermented functional foods. CRC Press, Boca Raton, FL.
- Molin G, et al. 1993. Numerical taxonomy of *Lactobacillus* spp. associated with healthy and diseased mucosa of the human intestines. J. Appl. Bacteriol. 74:314–323.
- Plengvidhya V, Breidt F, Jr, Lu Z, Fleming HP. 2007. DNA fingerprinting of lactic acid bacteria in sauerkraut fermentations. Appl. Environ. Microbiol. 73:7697–7702.
- Quevillon E, et al. 2005. InterProScan: protein domains identifier. Nucleic Acids Res. 33:W116–W120.
- Rud I, Solem C, Jensen PR, Axelsson L, Naterstad K. 2008. Co-factor engineering in lactobacilli: effects of uncoupled ATPase activity on metabolic fluxes in *Lactobacillus* (L.) *plantarum* and *L. sakei*. Met. Eng. 10:207– 215.
- 14. Shrago AW, Chassy BM, Dobrogosz WJ. 1986. Conjugal plasmid trans-

fer (pAMβ1) in *Lactobacillus plantarum*. Appl. Environ. Microbiol. **52**: 574–576.

- Siezen RJ, et al. 2012. Complete resequencing and reannotation of the Lactobacillus plantarum WCFS1 genome. J. Bacteriol. 194:195–196.
- Siezen RJ, et al. 2010. Phenotypic and genomic diversity of *Lactobacillus plantarum* strains isolated from various environmental niches. Environ. Microbiol. 12:758–773.
- 17. Siezen RJ, van Hylckama Vlieg JE. 2011. Genomic diversity and versatility of *Lactobacillus plantarum*, a natural metabolic engineer. Microb. Cell Fact. **10**(Suppl 1):S3.
- Sørvig E, et al. 2003. Construction of vectors for inducible gene expression in *Lactobacillus sakei* and *L. plantarum*. FEMS Microbiol. Lett. 229: 119–126.
- Sørvig E, Mathiesen G, Naterstad K, Eijsink VGH, Axelsson L. 2005. High-level, inducible gene expression in *Lactobacillus sakei* and *Lactobacillus plantarum* using versatile expression vectors. Microbiology 151: 2439–2449.
- Von Wright A, Axelsson L. 2011. Lactic acid bacteria: an introduction, p 1–16. *In* Lahtinen S, Ouwehand AC, Salminen S, Von Wright A (ed), Lactic acid bacteria, microbial and functional aspects, 4th ed. CRC Press, Boca Raton, FL.
- Wang Y, et al. 2011. Complete genome sequence of the probiotic Lactobacillus plantarum ST-III. J. Bacteriol. 193:313–314.
- Weinberg ZG, Muck RE, Weimer PJ. 2003. The survival of silage inoculant lactic acid bacteria in rumen fluid. J. Appl. Microbiol. 94:1066– 1071.
- Zhang ZY, et al. 2009. Complete genome sequence of *Lactobacillus plan*tarum JDM1. J. Bacteriol. 191:5020–5021.