Complete Genome Sequence of Lactobacillus acidophilus Strain ATCC 53544

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ABSTRACT

Here we present the complete genome sequence of Lactobacillus acidophilus ATCC 53544. The assembly contains 1,991,906 bp and is 99.7% similar to L. acidophilus NCFM. This strain was isolated from a rectal swab specimen of an infant and has previously been used as a feed supplement for animals.

The ATCC 53544 strain of Lactobacillus acidophilus (https://www.atcc.org/en/Products/All/53544.aspx) was originally isolated from a human infant rectal swab specimen (1). This strain has been shown to attach to human intestine cells, with improved adherence in the presence of calcium (1, 2). In addition, it has been found to inhibit growth of clinical toxic shock syndrome strains of Staphylococcus aureus, produce hydrogen peroxide, and degrade oxalate and bile salts (3–5).

The genome was sequenced on a Pacific Biosciences RS II sequencing platform using a 20-kb insert library and one single-molecule real-time (SMRT) sequencing cell. The genome assembly was carried out using the Pacific Biosciences Hierarchical Genome Assembly Process 2.0 (HGAP 2.0), and consensus polishing was performed with SMRTpipe Quiver. Glimmer v 3.02 was used to predict coding sequences (CDSs) and open reading frames (ORFs). Blastall was used for the alignment of ORFs and Blast2GO was used for annotation. rRNAs and tRNAs were predicted using RNAmmer 1.2 and tRNAscan-SE 1.4. The ATCC 53544 genome was aligned against other L. acidophilus genomes using progressiveMauve (6). The final assembly was composed of a single 1,991,906-bp chromosome with a mean G+C content of 34.7%. The genome contains one clustered regularly interspaced short palindromic repeat (CRISPR) array. The annotation predicted 1,892 CDSs, 12 rRNAs, and 61 tRNAs, and 1,482 of the annotated genes were categorized into 29 different functional Gene Ontology Consortium (GO) terms.

Comparative genome analysis revealed a 99.7% similarity with L. acidophilus NCFM (7), 99.9% similarity with L. acidophilus La-14 (8), and 98.6% similarity with L. acidophilus ATCC 4356 (9). In comparison with L. acidophilus NCFM, 245 single nucleotide polymorphisms (SNPs) and 121 gaps were found. One large chromosomal inversion was found (between 479,341 and 485,108 bp), where homologs of surface layer protein A (slpA), LBA0170, LBA0171, LBA0173, and slpB are in reverse complement orientation. Large regions that lack any detectible homology contain two 23S rRNAs and one 16S rRNA. Additionally, regions containing the majority of SNPs and gaps are near rearrangement sites, such as within gene homologs of a putative surface protein, LBA1654, previously identified as a potential strain-specific cell-surface protein in a differential BLAST analysis study in L. acidophilus (10), and hypothetical proteins LBA1655 and LBA1656. In comparison with the type strain L. acidophilus ATCC 4356, 201 SNPs and 219 gaps were found and several large chromosome inversions and rearrangements were identified. Of note were significant regions that lack detectable homology or many SNPs, including within the homologs of a putative cellobiose phosphotransferase, ERR203994_01916, a...
surface protein-encoding ERR203994_01916, slpB, and other surface proteins that are variable in *L. acidophilus* strains, including LBA1654. The *L. acidophilus* ATCC 53544 genome demonstrates similarities among the *L. acidophilus* strains sequenced so far and offers an opportunity to perform research to investigate how small genomic variations, in particular changes in genes encoding cell-surface proteins, may impact cell adherence, cell-host interaction, and other probiotic functionalities within the species.

**Accession number(s).** The genome sequence reported here has been deposited in GenBank under the accession number CP022449 and is a component of BioProject number PRJNA394684. The version described in this paper is CP022449.1.

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**REFERENCES**

1. Manfredi ET, Miller RE. August 1990. Novel strain of *Lactobacillus acidophilus*. US patent 4946791 A. Google Patents. https://www.google.com/patents/US4946791.
2. Manfredi ET, Miller RE. December 1990. Strains of *Lactobacillus* for enhancing feed conversion efficiency. US patent 4980164 A. Google Patents. https://www.google.com/patents/US4980164.
3. Elkins CA, Muñoz ME, Mullis LB, Stingley RL, Hart ME. 2008. *Lactobacillus*-mediated inhibition of clinical toxic shock syndrome *Staphylococcus aureus* strains and its relation to acid and peroxide production. Anaerobe 14:261–267. https://doi.org/10.1016/j.anaerobe.2008.08.003.
4. Cho JG, Gebhart CJ, Furrow E, Lulich JP. 2015. Assessment of in vitro oxalate degradation by *Lactobacillus* species cultured from veterinary probiotics. Am J Vet Res 76:801–806. https://doi.org/10.2460/ajvr.76.9.801.
5. Moser SA, Savage DC. 2001. Bile salt hydrolase activity and resistance to toxicity of conjugated bile salts are unrelated properties in lactobacilli. Appl Environ Microbiol 67:3476–3480. https://doi.org/10.1128/AEM.67.8.3476-3480.2001.
6. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147.
7. Altermann E, Russell WM, Azcarate-Peril MA, Barrangou R, Buck BL, McAuliffe O, Souther N, Dobson A, Duong T, Callanan M, Lick S, Hamrick A, Cano R, Klaenhammer TR. 2005. Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. Proc Natl Acad Sci U S A 102:3906–3912. https://doi.org/10.1073/pnas.0409188102.
8. Stahl B, Barrangou R. 2013. Complete genome sequence of probiotic strain *Lactobacillus acidophilus* la-14. Genome Announc 1(3):e00376-13. https://doi.org/10.1128/genomeA.00376-13.
9. Palomino MM, Allievi MC, Fina Martin J, Waehner PM, Prado Acosta M, Sanchez Rivas C, Ruzal SM. 2015. Draft genome sequence of the probiotic strain *Lactobacillus acidophilus* ATCC 4356. Genome Announc 3(1):e01421-14. https://doi.org/10.1128/genomeA.01421-14.
10. Altermann E, Klaenhammer TR. 2011. Group-specific comparison of four lactobacilli isolated from human sources using differential blast analysis. Genes Nutr 6:319–340. https://doi.org/10.1007/s12263-010-0191-9.