genomeA
Journals.ASM.org

Draft Genome Sequence of the Probiotic Strain *Lactobacillus acidophilus* ATCC 4356

Maria Mercedes Palomino, Mariana C. Allievi, Joaquina Fina Martin, Pablo M. Waehner, Mariano Prado Acosta, Carmen Sanchez Rivas, Sandra M. Ruzal

Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, IQUIBICEN-CONICET, Buenos Aires, Argentina

M.M.P. and M.C.A. contributed equally to this work.

We present the 1,956,699-bp draft genome sequence of *Lactobacillus acidophilus* strain ATCC 4356. Comparative genomic analysis revealed 99.96% similarity with *L. acidophilus* NCFM NC_006814.3 and 99.97% with La-14 NC_021181.2 genomes.

Received 3 December 2014 **Accepted** 4 December 2014 **Published** 15 January 2015

Citation 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. doi:10.1128/genomeA.01421-14.

Address correspondence to Sandra M. Ruzal, sandra@qb.fcen.uba.ar.

The original *Lactobacillus acidophilus* strain ATCC 4356 was isolated in 1900 (1, 2) from human infant feces. This strain is available from the American Type Culture Collection (http://www.atcc.org/Products/All/4356.aspx). *L. acidophilus* ATCC 4356 is an important inhabitant of the gastrointestinal tract with reported probiotic properties (3–5). The characterization of its probiotic attributes is a relevant goal. In our lab, we are interested in the cell envelope structure (6, 7), and we have found candidate probiotic functions for its surface-layer protein (8–10).

The strain did not contain any plasmid but did contain a unique genome. The genome sequence was obtained using a whole-genome shotgun strategy with a 454 GS Titanium pyrosequencer at the Instituto de Agrobiotecnología Rosario (INDEAR), Argentina. Assembly was done using 454 Newbler version 2.6, with a genome coverage of 20×. This assembly generated 20 scaffolds using Mauve version 2.3 and the *L. acidophilus* NCFM genome as a template. The draft genome is 1,956,699 bp in length, and the G+C content is 34.6%. Genomic annotations were assigned automatically by the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) with some minor additions from Rapid Annotations using Subsystems Technology (RAST) (11, 12). The annotation predicted 1,977 coding sequences (CDSs) and 62 structural RNAs (59 tRNAs). A total of 506 CDSs (25.6%) were classified as hypothetical proteins. According to RASTtk, the annotation identified 903 CDSs (46%) into RAST subsystems. The genome also contains 1 CRISPR array.

Comparative genomic analysis revealed 99.96% similarity with *L. acidophilus* NCFM NC_006814.3 (13) and 99.97% similarity with La-14 NC_021181.2 (14); the genomes also shared high synteny. BLASTn analysis of the 29 sequences of this strain deposited in GenBank shows that 22 out of 29 presented between 99 and 100% identity. Differences would rely on the methods employed since higher identity was observed for the more recent nucleotide sequences.

A differential feature never annotated before in *Lactobacillus acidophilus* strains was the presence of a coding sequence for chitinase activity (EC 2.7.1.69, NH13_08655). In fact, several chitin and *N*-acetylglucosamine utilization functions were predicted by RASTtk, particularly the PTS system for *N*-acetylglucosamine-specific IIABC components (NH13_02050), as well as the *N*-acetyl-D-glucosamine ABC transport system, permease protein (NH13_08155). Mechanisms of the enzymes degrading *N*-acetylglucosamine (15) are predicted: NagB, GlcN6P deaminase (NH13_09645) and NagA, GlcNAc6P deacetylase (NH13_00815). A transcriptional regulator of *N*-acetylglucosamine utilization (NH13_02010) from the GntR family was also found. We confirm their functionality: cells were able to grow in chitin as the sole carbon source and in plates containing chitin and stained with calcofluor, and a degradation halo was observed. Moreover, the hydrolytic activity was found in the cell wall fractions. The presence of chitinase might constitute a system for scavenging mucosa-derived carbohydrates, as well as an adherence factor, since GlcNAc is found in many human glycoproteins at mucosal surfaces and probably represents an adaptation for development at the intestinal mucosal niche.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JRUT00000000. The version described in this paper is version JRUT01000000.

ACKNOWLEDGMENT

This work was supported by "Agencia Nacional Promoción Científica y Tecnológica" grant PICT2012-0789

REFERENCES

1. **Moro E.** 1900. Ueber den *Bacillus acidophilus*. Jahrb Kinderheilk **52:** 38–55.
2. **Moro E.** 1900. Ueber die nach Gram farbbaren Bacillen des Säuglingsstuhles. Wien Klin Wochenschr **13:**114–115.
3. **Chen X, Xu J, Shuai J, Chen J, Zhang Z, Fang W.** 2007. The S-layer proteins of *Lactobacillus crispatus* strain ZJ001 is responsible for competitive exclusion against *Escherichia coli* O157:H7 and *Salmonella typhimurium*. Int J Food Microbiol **115:**307–312. http://dx.doi.org/10.1016/ j.ijfoodmicro.2006.11.007.
4. **Foligne B, Nutten S, Grangette C, Dennin V, Goudercourt D, Poiret S, Dewulf J, Brassart D, Mercenier A, Pot B.** 2007. Correlation between *in vitro* and *in vivo* immunomodulatory properties of lactic acid bacteria.

World J Gastroenterol **13:**236–243. http://dx.doi.org/10.3748/wjg.v13.i2.236.

5. **Resta-Lenert S, Barrett KE.** 2003. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut **52:**988–997. http://dx.doi.org/10.1136/gut.52.7.988.
6. **Palomino MM, Allievi MC, Gründling A, Sanchez-Rivas C, Ruzal SM.** 2013. Osmotic stress adaptation in *Lactobacillus casei* BL23 leads to structural changes in the cell wall polymer lipoteichoic acid. Microbiology **159:** 2416–2426. http://dx.doi.org/10.1099/mic.0.070607-0.
7. **Piuri M, Sanchez-Rivas C, Ruzal SM.** 2005. Cell wall modifications during osmotic stress in *Lactobacillus casei*. J Appl Microbiol **98:**84–95. http://dx.doi.org/10.1111/j.1365-2672.2004.02428.x.
8. **Martínez MG, Acosta MP, Candurra NA, Ruzal SM.** 2012. Layer proteins of *Lactobacillus acidophilus* inhibits JUNV infection. Biochem Biophys Res Commun **422:**590–595. http://dx.doi.org/10.1016/ j.bbrc.2012.05.031.
9. **Prado Acosta M, Palomino MM, Allievi MC, Sanchez Rivas C, Ruzal SM.** 2008. Murein hydrolase activity in the surface layer of *Lactobacillus acidophilus* ATCC 4356. Appl Environ Microbiol **74:**7824–7827. http://dx.doi.org/10.1128/AEM.01712-08.
10. **Prado-Acosta M, Ruzal SM, Allievi MC, Palomino MM, Sanchez Rivas C.** 2010. Synergistic effects of the *Lactobacillus acidophilus* surface layer and nisin on bacterial growth. Appl Environ Microbiol **76:**974–977. http://dx.doi.org/10.1128/AEM.01427-09.
11. **Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O.** 2008. Toward an online repository of standard operating procedures (SOPs) for (meta)genomic annotation. OMICS **12:**137–141. http://dx.doi.org/10.1089/omi.2008.0017.
12. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O.** 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics **9:**75. http://dx.doi.org/10.1186/1471-2164-9-75.
13. **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. http://dx.doi.org/10.1073/pnas.0409188102.
14. **Stahl B, Barrangou R.** 2013. Complete genome sequence of probiotic strain *Lactobacillus acidophilus* La-14. Genome Announc **1**(3):e00376-13. http://dx.doi.org/10.1128/genomeA.00376-13.
15. **Bidart GN, Rodríguez-Díaz J, Monedero V, Yebra MJ.** 2014. A unique gene cluster for the utilization of the mucosal and human milk-associated glycans galacto-*N*-biose and lacto-*N*-biose in *Lactobacillus casei*. Mol Microbiol **93:**521–538. http://dx.doi.org/10.1111/mmi.12678.