

## Genome Sequence of the Probiotic Strain Lactobacillus rhamnosus (Formerly Lactobacillus casei) LOCK900

## Tamara Aleksandrzak-Piekarczyk, Anna Koryszewska-Bagińska, Jacek Bardowski

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

*Lactobacillus rhamnosus* LOCK900 fulfills the criteria required for probiotic strains. In this study, we report a whole-genome sequence of this isolate and compare it with other *L. rhamnosus* complete genome sequences already published.

Received 18 July 2013 Accepted 22 July 2013 Published 15 August 2013

Citation Aleksandrzak-Piekarczyk T, Koryszewska-Bagińska A, Bardowski J. 2013. Genome sequence of the probiotic strain Lactobacillus rhamnosus (formerly Lactobacillus casei) LOCK900. Genome Announc. 1(4):e00640-13. doi:10.1128/genomeA.00640-13.

**Copyright** © 2013 Aleksandrzak-Piekarczyk et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license. Address correspondence to Tamara Aleksandrzak-Piekarczyk, tamara@ibb.waw.pl.

actobacillus rhamnosus strain LOCK900 (formerly Lactobacil-

Literative Control of the Technical University, Lódz, Poland. The new species affinity of this strain was identified in this study based on the sequences of genomic markers, such as 16S rRNA, *rpoA*, and *pheS* genes (1).

It has been shown that *L. rhamnosus* LOCK900 fulfills the criteria required for probiotic strains, such as resistance to high concentrations of bile salts and low pH (2, 3). Also, its ability to adhere to cells of the Caco-2 epithelial cell line has been reported (4). In addition, its antiallergic potential has been manifested by induction of T<sub>h</sub>1 cytokines (interleukin 12 [IL-12], IL-18, gamma interferon [IFN- $\gamma$ ], and tumor necrosis factor alpha [TNF- $\alpha$ ]) and regulatory transforming growth factor  $\beta_1$  (TGF- $\beta_1$ ) in blood cell cultures of allergic children (5). Lastly, no pro-allergic cytokines were found after bacterial stimulation (5).

The whole genome of *L. rhamnosus* LOCK900 was sequenced using the GS FLX Titanium platform (Roche). Paired and unpaired reads obtained with 81-fold genomic coverage were assembled using Newbler *de novo* assembler (Roche) software. The final circularized genome molecule of 2,883,376 bp with a G+C content of 46.8% was deciphered.

Genome annotation was performed by merging the result from the RAST (Rapid Annotation using Subsystem Technology) server (6) and tRNAscan-SE (7) and followed by manual inspection, which predicted 2,838 coding sequences (CDS), 59 tRNAs, and 5 rRNA loci. Of the total number of CDS found, approximately 760 are hypothetical proteins with unknown functions. No plasmids were found in this isolate, which is relatively common for lactobacilli and different from lactococci (8).

The analysis retrieved from the RAST server revealed 310 subsystems existing in *L. rhamnosus* LOCK900 and revealed the absence of subsystem features for photosynthesis, iron acquisition and metabolism, motility, and chemotaxis, which has also been observed in all other completely sequenced genomes of this species (9–11). Due to the genome size reduction compared with other fully sequenced strains of *L. rhamnosus*, LOCK900 displaces a reduced number of genes distributed in individual categories, particularly carbohydrates, cell wall and capsule, virulence, disease, and defense. *L. rhamnosus* LOCK900 also showed a lack of many genes distributed in the categories of phages and prophages; however, the analysis performed via the PHAST (Phage Search Tool) server (12) indicated the existence of one intact and one incomplete prophage within its genome. On the other hand, despite the LOCK900 reduced genome, many genes are more abundantly represented, mostly in the categories of protein metabolism, RNA metabolism and regulation, and cell signaling.

Comparative genomic analysis of *L. rhamnosus* LOCK900 with four published *L. rhamnosus* complete genome sequences showed it has the greatest similarity to *L. rhamnosus* Lc705 and *L. rhamnosus* ATCC 8530 and less similarity to the probiotic isolate *L. rhamnosus* GG. In comparison to *L. rhamnosus* Lc705 and ATCC 8530, this analysis showed the presence of CRISPR-associated proteins and the existence of a complete type II restriction-modification (RM) system consisting of a restriction endonuclease and modification methylase EcoRV in the *L. rhamnosus* LOCK900 genome.

**Nucleotide sequence accession number.** The complete sequence of the *L. rhamnosus* LOCK900 genome is deposited in GenBank under the accession number CP005484.

## ACKNOWLEDGMENTS

Genome sequencing was done at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences.

This research was supported by the Technical University of Lodz grant from the National Centre for Research and Development no. 12/P01/2010/10.

## REFERENCES

- Naser SM, Dawyndt P, Hoste B, Gevers D, Vandemeulebroecke K, Cleenwerck I, Vancanneyt M, Swings J. 2007. Identification of lactobacilli by *pheS* and *rpoA* gene sequence analyses. Int. J. Syst. Evol. Microbiol. 57:2777–2789.
- Cukrowska B, Motyl I, Kozáková H, Schwarzer M, Górecki RK, Klewicka E, Ŝliżewska K, Libudzisz Z. 2009. Probiotic *Lactobacillus* strains: in vitro and in vivo studies. Folia Microbiol. (Praha) 54:533–537.
- 3. Motyl I. 2002. Ph.D. thesis. Technical University of Lodz, Lódz, Poland.
- Marewicz E, Moneta J, Motyl I, Heczko P, Libudzisz Z. 2000. Comparison of adherence to Caco-2 human epithelial cell line of *Lactobacillus* and *Bifidobacterium* strains of various origin: human, animal and vegetal. Med. Sci. Prompt. 54(Suppl. 3):34.

- Cukrowska B, Rosiak I, Klewicka E, Motyl I, Schwarzer M, Libudzisz Z, Kozakova H. 2010. Impact of heat-inactivated *Lactobacillus casei* and *Lactobacillus paracasei* strains on cytokine responses in whole blood cell cultures of children with atopic dermatitis. Folia Microbiol. (Praha) 55: 277–280.
- Aziz R, Bartels D, Best A, DeJongh M, Disz T, Edwards R, Formsma K, Gerdes S, Glass E, Kubal M, Meyer F, Olsen G, Olson R, Osterman A, Overbeek R, McNeil L, Paarmann D, Paczian T, Parrello B, Pusch G, 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. doi:10.1186/1471-2164-9-75.
- 7. Schattner P, Brooks AN, Lowe TM. 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res. 33:W686–W689. doi:10.1093/nar/gki366.
- Górecki RK, Koryszewska-Bagińska A, Gołębiewski M, Żylińska J, Grynberg M, Bardowski JK. 2011. Adaptative potential of the *Lactococcus lactis* IL594 strain encoded in its 7 plasmids. PLoS One 6:e22238. doi:10. 1371/journal.pone.0022238.
- 9. Kankainen M, Paulin L, Tynkkynen S, von Ossowski I, Reunanen J, Partanen P, Satokari R, Vesterlund S, Hendrickx APA, Lebeer S, De Keersmaecker SCJ, Vanderleyden J, Hämäläinen T, Laukkanen S, Salovuori N, Ritari J, Alatalo E, Korpela R, Mattila-Sandholm T, Lassig A, Hatakka K, Kinnunen KT, Karjalainen H, Saxelin M, Laakso K, Surakka A, Palva A, Salusjärvi T, Auvinen P, de Vos WM. 2009. Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human-mucus binding protein. Proc. Natl. Acad. Sci. U. S. A. 106: 17193–17198.
- Morita H, Toh H, Oshima K, Murakami M, Taylor TD, Igimi S, Hattori M. 2009. Complete genome sequence of the probiotic *Lactobacillus rham-nosus* ATCC 53103. J. Bacteriol. 191:7630–7631.
- 11. Pittet V, Ewen E, Bushell BR, Ziola B. 2012. Genome sequence of *Lactobacillus rhamnosus* ATCC 8530. J. Bacteriol. **194**:726.
- Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. Nucleic Acids Res. 39:W347–W352. doi:10.1093/nar /gkr485.