



Multiple Genome Sequences of *Lactobacillus pentosus* Strains Isolated from Biofilms on the Skin of Fermented Green Table Olives

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ABSTRACT The draft genome sequences of five *Lactobacillus pentosus* strains isolated from biofilms on the skin of green table olives are presented here. These genome sequences will assist in revealing the potential probiotic properties of these strains, as the intake of fermented olives implicates the passage of millions of *Lactobacillus* spp. throughout a consumer's gastrointestinal tract.

Lactobacillus pentosus is the most important microorganism responsible for the fermentation of olives (1–3). In the past, it was assumed that this species exclusively appeared in a planktonic state (4, 5), but we now know that it makes biofilms on the skin of fermented olives (6, 7). Therefore, billions of *L. pentosus* cells would be delivered to the human gastrointestinal tract (GIT) with the intake of olives (8). Given the probiotic potential of *L. pentosus* (9), the fermented olives could be further considered to be a vehicle for the entry of beneficial microorganisms into the GIT. We report here the draft genome sequence of *L. pentosus*, isolated from biofilms on the skin of traditional fermented olives.

To recover *L. pentosus* from biofilms, a stomacher method was used (6). Detached biofilms were spread onto de Man-Rogosa-Sharpe (MRS) plates, and isolated colonies were identified at the molecular level as *L. pentosus* (10). To obtain genomic DNA, a modification of the “salting-out” procedure was followed (11). Genome libraries for DNA sequencing were constructed using a TruSeq DNA PCR-free library preparation kit (Illumina, Inc.), with an insert size of 350 bp. The sequencing process was carried out at Macrogen, Inc. (Seoul, Republic of Korea) using a HiSeq Illumina platform, obtaining paired-end sequencing reads with 2 × 101-bp read lengths. Assembly was performed using Velvet 1.2.10 (12), optimizing parameters with VelvetOptimiser 2.2.5 (12).

The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (13) was used to annotate the strains, and it was completed using the following protocol: protein-coding genes were predicted using Prodigal version 2.6.3 (14), and then they were functionally annotated by Sma3s v2 using UniProt bacteria (15). To annotate noncoding genes, Infernal 1.1.2 (16) was used with the Rfam database 13.0 (17). To estimate the number of plasmids appearing in each strain, the contig sequences were compared to all the plasmid sequences from *Lactobacillus* species available in the RefSeq database using BLASTN and 90% for both identity and coverage.

The genomes of all the strains are split into around 100 contigs, having a mean length of 3,795,672 bp, with an estimated G+C content of 45.9%. The numbers of predicted genes were similar in all the cases (Table 1).

The functional annotation was used to discover genes involved in specific functions, and we also performed a similarity search using BLASTP with a threshold of 80% in both identity and query coverage, using *Lactobacillus* sequences from the protein database UniProtKB

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TABLE 1 Genome information and GenBank accession numbers of five *Lactobacillus pentosus* strains isolated from biofilms on the skin of fermented Spanish-style green olives

Strain	BioSample accession no.	No. of reads	Avg coverage (×)	Assembly size (bp)	No. of contigs	N_{50} (bp)	G+C content (%)	No of protein-coding genes	No. of plasmids	No. of tRNAs	No. of rRNAs
IG8	SAMN10112407	41,135,818	2,790.70	3,791,593	99	312,635	45.91	3,450	6	79	24
IG9	SAMN10112443	34,194,869	957.90	3,787,967	99	278,654	45.91	3,447	6	81	16
IG10	SAMN10112444	36,357,603	972.21	3,811,295	121	98,672	45.95	3,432	7	78	12
IG11	SAMN10112445	41,832,814	2,871.40	3,790,820	107	312,529	45.91	3,448	6	78	20
IG12	SAMN10112446	47,220,444	1,456.30	3,796,685	81	269,556	45.90	3,459	6	80	16

(18). After that, four strains showed two copies of the *luxS* gene, which plays a key role in the synthesis of the universal bacterial communicator autoinductor-2 (19). Also, a high number of genes involved in bacteriocin and exopolysaccharide (EPS) production was found. Interestingly, several genes encoding MucBP proteins, which could play an important role in microbe-eukaryote cell adhesion (20), were also found. Taking into account the importance of all these genes in the probiotic features of lactic acid bacteria, the genome sequences reported here will aid in future research into the probiotic potential of *L. pentosus*.

Data availability. The genome sequences of all the strains have been deposited under NCBI BioProject number [PRJNA492883](#), and the BioSample accession numbers are listed in Table 1. The reads have been deposited in the NCBI Sequence Read Archive (SRA) under the accession numbers [SRX5116733](#) to [SRX5116737](#), and the assemblies have been deposited in GenBank under the accession numbers [RDCL00000000](#), [RDCK00000000](#), [RDCJ00000000](#), [RDCI00000000](#), and [RDCH00000000](#).

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REFERENCES

- Benítez-Cabello A, Bautista-Gallego J, Garrido-Fernández A, Rantsiou K, Coccolin L, Jiménez-Díaz R, Arroyo-López FN. 2016. RT-PCR-DGGE analysis to elucidate the dominant bacterial species of industrial Spanish-style green table olive fermentations. *Front Microbiol* 7:1291. <https://doi.org/10.3389/fmicb.2016.01291>.
- Pérez-Díaz I, Breidt F, Buescher RW, Arroyo-López FN, Jiménez-Díaz R, Garrido-Fernández A, Bautista-Gallego J, Yoon SS, Johanningsmeier SD. 2013. Chapter 51: fermented and acidified vegetables. In Downes FP, Ito K (ed), *Compendium of methods for the microbial examination of foods*, 4th ed. American Public Health Association, Washington, DC.
- Ruiz-Barba JL, Ríos-Sánchez RM, Fedriani-Iriso C, Olías JM, Ríos JL, Jiménez-Díaz R. 1990. Bactericidal effect of phenolic compounds from green olives on *Lactobacillus plantarum*. *Syst Appl Microbiol* 13:199–205. [https://doi.org/10.1016/S0723-2020\(11\)80170-0](https://doi.org/10.1016/S0723-2020(11)80170-0).
- Ruiz-Barba JL, Cathcart DP, Warner PJ, Jiménez-Díaz R. 1994. Use of *Lactobacillus plantarum* LPCO10, a bacteriocin producer, as a starter culture in Spanish-style green olive fermentation. *Appl Environ Microbiol* 60:2059–2064. <https://aem.asm.org/content/60/6/2059>.
- Hurtado A, Reguant C, Bordons A, Rozès N. 2010. Evaluation of a single and combined inoculation of a *Lactobacillus pentosus* starter for processing cv. *Arbequina* natural green olives. *Food Microbiol* 27:731–740. <https://doi.org/10.1016/j.fm.2010.03.006>.
- Domínguez-Manzano J, Olmo-Ruiz C, Bautista-Gallego J, Arroyo-López FN, Garrido-Fernández A, Jiménez-Díaz R. 2012. Biofilm formation on abiotic and biotic surfaces during Spanish style green table olive fermentation. *Int J Food Microbiol* 157:230–238. <https://doi.org/10.1016/j.ijfoodmicro.2012.05.011>.
- Nychas G-JE, Panagou EZ, Parker ML, Waldron KW, Tassou CC. 2002. Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. *Lett Appl Microbiol* 34:173–177. <https://doi.org/10.1046/j.1472-765x.2002.01077.x>.
- Arroyo-López FN, García-García P, Rodríguez-Gómez F, Garrido-Fernández A. 2016. Olives: types and consumption, p 167–170. In Caballero B, Finglas PM, Toldrá F (ed), *Encyclopedia of food and health*. Elsevier Ltd., Waltham, MA.
- Rodríguez-Gómez F, Bautista-Gallego J, Arroyo-López FN, Romero-Gil V, Jiménez-Díaz R, Garrido-Fernández A, García-García P. 2013. Table olive fermentation with multifunctional *Lactobacillus pentosus* strains. *Food Control* 34:96–105. <https://doi.org/10.1016/j.foodcont.2013.04.010>.
- Torriani S, Felis GE, Dellaglio F. 2001. Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR assay with *recA* gene-derived primers. *Appl Environ Microbiol* 67:3450–3454. <https://doi.org/10.1128/AEM.67.8.3450-3454.2001>.
- Martín-Platero AM, Valdivia E, Maqueda M, Martínez-Bueno M. 2007. Fast, convenient, and economical method for isolating genomic DNA from lactic acid bacteria using a modification of the protein “salting-out” procedure. *Anal Biochem* 366:102–104. <https://doi.org/10.1016/j.ab.2007.03.010>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.

14. Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <https://doi.org/10.1186/1471-2105-11-119>.
15. Casimiro-Soriguer CS, Muñoz-Mérida A, Pérez-Pulido AJ. 2017. Sma3s: a universal tool for easy functional annotation of proteomes and transcriptomes. *Proteomics* 17:1700071. <https://doi.org/10.1002/pmic.201700071>.
16. Nawrocki EP, Eddy SR. 2013. Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics* 29:2933–2935. <https://doi.org/10.1093/bioinformatics/btt509>.
17. Kalvari I, Argasinska J, Quinones-Olvera N, Nawrocki EP, Rivas E, Eddy SR, Bateman A, Finn RD, Petrov AI. 2018. Rfam 13.0: shifting to a genome-centric resource for non-coding RNA families. *Nucleic Acids Res* 46: D335–D342. <https://doi.org/10.1093/nar/gkx1038>.
18. The UniProt Consortium. 2018. UniProt: the universal protein knowledge-base. *Nucleic Acids Res* 46:2699. <https://doi.org/10.1093/nar/gky092>.
19. Schauder S, Shokat K, Surette MG, Bassler BL. 2001. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol Microbiol* 41:463–476. <https://doi.org/10.1046/j.1365-2958.2001.02532.x>.
20. Boekhorst J, Helmer Q, Kleerebezem M, Siezen RJ. 2006. Comparative analysis of proteins with a mucus-binding domain found exclusively in lactic acid bacteria. *Microbiology* 152:273–280. <https://doi.org/10.1099/mic.0.28415-0>.