

Draft Genome Sequence of *Pediococcus parvulus* 2.6, a Probiotic β -Glucan Producer Strain

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We report here the draft genome sequence of the probiotic *Pediococcus parvulus* 2.6, a lactic acid bacterial strain isolated from ropy cider. The bacterium produces a prebiotic and immunomodulatory exopolysaccharide, and this is the first strain of the *P. parvulus* species whose genome has been characterized.

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We report here the draft genome sequence of *Pediococcus parvulus* 2.6 (formerly *Pediococcus damnosus*), a lactic acid bacterium isolated from ropy cider (1). This is the first strain of *P. parvulus* whose genome has been characterized. The 2.6 strain produces the immunomodulatory exopolysaccharide 2-substituted (1,3)- β -D-glucan (2), which is synthesized by a limited number of bacteria and confers probiotic properties to the producing strains. This polysaccharide differs from the β -glucans produced by plants and other microorganisms. The synthesis of 2-substituted (1,3)- β -D-glucan by *P. parvulus* 2.6 is controlled by a single heterotransmembrane glucosyltransferase (GTF), which polymerizes glucosyl residues from UDP glucose (3–5). Some of the conditions that influence the homopolysaccharide (HoPS) synthesis by *P. parvulus* 2.6, as well as the enzymatic activities involved in sugar metabolism in this strain, have been examined (6, 7). The physicochemical properties of the HoPS have also been determined, and nuclear magnetic resonance (NMR) analysis showed that the same HoPS was synthesized, irrespective of the sugar source used for growth (8). The molecular masses of these β -glucans are high ($>10^6$ Da), and their rheological properties showed that they have potential utility as biothickeners (8). An oat-based product fermented by *P. parvulus* 2.6 showed improved rheology (9), thereby decreasing the need for added stabilizers and texturizers.

The potential of *P. parvulus* 2.6 as a probiotic strain has also been examined; it resists gastrointestinal stress, adheres to Caco-2 cells, and its HoPS reduces the production of inflammatory cytokines by polarized macrophages (10). We have also shown that *P. parvulus* HoPS improves the growth and viability of probiotic microorganisms, as well as their adhesion to human enterocytes (11). The 2-substituted (1,3)- β -D-glucan increases *in vitro* the ratio of interleukin 10 (IL10) (anti-inflammatory) to tumor necrosis factor alpha (TNF α) (inflammatory) in human macrophages and decreases the levels of the proinflammatory IL8 in human intes-

tine organ cultures (reference 12 and Notararigo S., Antolin M., Guarner F., López P., unpublished data).

An oat-based product fermented by *P. parvulus* 2.6 had a bifidogenic effect and decreased serum cholesterol levels in humans (13). Possibly, the 2-substituted (1,3)- β -D-glucan has a synergistic effect with the hypocholesterolemic action of oat β -glucans. Finally, *P. parvulus* 2.6 displays antibacterial activity against several bacterial species (14), including natural contaminants in oats, a property that reduces the need for chemical preservatives and improves the functionality of the final product.

Two micrograms of genomic DNA was subjected to library preparation using the TruSeq DNA sample prep kit FC-121-1001, according to the manufacturer's instructions. Whole-genome sequencing used the Illumina GAIIx at the Genomics Research Centre (Fiorenzuola d'Arda, Italy). A total of 26,018,224 paired-end reads (2×110 -bp length) were assembled into 115 contigs. The genome was calculated to be 2,236,754 long. The size of the shortest contig was 206 bp, while the length of the longest contig was 171,226 bp. The genome sequence was annotated by the NCBI Prokaryotic Genomes Annotation Pipeline. A total of 2,241 genes were predicted to encode 2,069 proteins, three rRNAs, 60 tRNAs, and four noncoding RNAs (ncRNAs), and 105 are pseudogenes.

Accession number(s). The complete genome of *P. parvulus* 2.6 has been deposited at DDBJ/EMBL/GenBank under accession number [LXND000000000](https://www.ncbi.nlm.nih.gov/nuclink/LXND000000000).

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REFERENCES

1. Fernández K, Dueñas M, Irastorza A, Bilbao A, del Campo G. 1995. Characterization and DNA plasmid analysis of ropy *Pediococcus* spp. strains isolated from Basque Country ciders. *J Food Protect* 59:35–40.
2. Dueñas-Chasco MT, Rodríguez-Carvajal MA, Mateo PT, Franco-Rodríguez G, Espartero J, Irastorza-Iribas A, Gil-Serrano AM. 1997. Structural analysis of the exopolysaccharide produced by *Pediococcus damnosus* 2.6. *Carbohydr Res* 303:453–458. [http://dx.doi.org/10.1016/S0008-6215\(97\)00192-4](http://dx.doi.org/10.1016/S0008-6215(97)00192-4).
3. Werning ML, Ibarburu I, Dueñas MT, Irastorza A, Navas J, López P. 2006. *Pediococcus parvulus* *gtf* gene encoding the GTF glycosyltransferase and its application for specific PCR detection of β -D-glucan-producing bacteria in foods and beverages. *J Food Prot* 69:161–169.
4. Werning ML, Corrales MA, Prieto A, de Palencia PF, Navas J, López P. 2008. Heterologous expression of a position 2-substituted (1 \rightarrow 3)- β -D-glucan in *Lactococcus lactis*. *Appl Environ Microbiol* 74:5259–5262. <http://dx.doi.org/10.1128/AEM.00463-08>.
5. Werning ML, Pérez-Ramos A, Fernández de Palencia P, Mohedano ML, Dueñas MT, Prieto A, López P. 2014. A specific immunological method to detect and quantify bacterial 2-substituted (1,3)- β -D-glucan. *Carbohydr Polym* 113:39–45. <http://dx.doi.org/10.1016/j.carbpol.2014.06.072>.
6. Velasco S, Arsköld E, Paese M, Grage H, Irastorza A, Rådström P, van Niel EW. 2006. Environmental factors influencing growth of and exopolysaccharide formation by *Pediococcus parvulus* 2.6. *Int J Food Microbiol* 111:252–258. <http://dx.doi.org/10.1016/j.ijfoodmicro.2006.06.008>.
7. Velasco SE, Yebra MJ, Monedero V, Ibarburu I, Dueñas MT, Irastorza A. 2007. Influence of the carbohydrate source on β -glucan production and enzyme activities involved in sugar metabolism in *Pediococcus parvulus* 2.6. *Int J Food Microbiol* 115:325–334. <http://dx.doi.org/10.1016/j.ijfoodmicro.2006.12.023>.
8. Velasco SE, Areizaga J, Irastorza A, Dueñas MT, Santamaria A, Muñoz ME. 2009. Chemical and rheological properties of the β -glucan produced by *Pediococcus parvulus* 2.6. *J Agric Food Chem* 57:1827–1834. <http://dx.doi.org/10.1021/jf803065w>.
9. Mårtensson O, Staaf J, Dueñas-Chasco MT, Irastorza A, Oste R, Holst O. 2002. A fermented ropy non-dairy oat product based on the exopolysaccharide-producing strain *Pediococcus damnosus* 2.6. *Adv Food Sci* 24: 4–11. <http://dx.doi.org/10.1016/j.nutres.2005.03.004>.
10. de Palencia PF, Werning ML, Sierra-Filardi E, Dueñas MT, Irastorza A, Corbí AL, López P. 2009. Probiotic properties of the 2-substituted (1,3)- β -D-glucan producing *Pediococcus parvulus* 2.6. *Appl Environ Microbiol* 75:4887–4891. <http://dx.doi.org/10.1128/AEM.00394-09>.
11. Russo P, López P, Capozzi V, de Palencia PF, Dueñas MT, Spano G, Fiocco D. 2012. Beta-glucans improve growth, viability and colonization of probiotic microorganisms. *Int J Mol Sci* 13:6026–6039. <http://dx.doi.org/10.1128/AEM.00394-09>.
12. Notararigo S, de las Casas-Engel M, Fernández de Palencia P, Corbí AL, López P. 2014. Immunomodulation of human macrophages and myeloid cells by 2-substituted (1,3)- β -D-glucan from *P. parvulus* 2.6. *Carbohydr Polym* 112:109–113. <http://dx.doi.org/10.1016/j.carbpol.2014.05.073>.
13. Mårtensson O, Björklund M, Lambo AM, Dueñas-Chasco M, Irastorza A, Holst O, Norin E, Welling G, Öste R, Önning G. 2005. Fermented ropy, oat-based products reduce cholesterol levels and stimulate the bifidobacteria flora in humans. *Nutr Res* 25:429–442. <http://dx.doi.org/10.1016/j.nutres.2005.03.004>.
14. Immerstrand T, Paul CJ, Rosenquist A, Deraz S, Mårtensson OB, Ljungh A, Blücher A, Öste R, Holst O, Karlsson EN. 2010. Characterization of the properties of *Pediococcus parvulus* for probiotic or protective culture use. *J Food Prot* 73:960–967.