

Draft Genome Sequence of Probiotic Strain *Pediococcus acidilactici* MA18/5M

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***Pediococcus acidilactici* MA18/5M is a commercially available probiotic that is widely used in swine, poultry, aquaculture feeds, and human dietary supplements. We prepared a genome sequence for this strain consisting of 2 scaffolds totaling 1,992,928 bases including gaps for a total of 3,346 bases and a G+C content of 42%.**

Pediococcus acidilactici strain R1001, previously known as EQ-01, was deposited at the Institut Pasteur Collection Nationale de Cultures de Micro-organismes as MA18/5M on 22 March 1991. It was isolated in France from natural-pasture Gramineae, initially for use in the animal feed industry. The strain has been extensively used as a probiotic in a number of commercial products for pigs, poultry, and aquaculture and eventually for human dietary supplements (2, 3, 4).

The sample was prepared for sequencing by growing the *P. acidilactici* MA18/5M strain anaerobically overnight at 37°C in de Man-Rogosa-Sharpe broth (Oxoid; catalog no. CM0361). Genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen; catalog no. 69504) according to the manufacturer's recommended protocol for Gram-positive bacteria. The quantity of DNA obtained was determined using an ND1000 Nanodrop spectrophotometer; typically, 1 to 5 µg of DNA was sent to Genome Quebec (Montréal, Quebec, Canada) for sequencing per their specifications.

The genome was sequenced with the 454 GS 20, yielding 319,611 reads with an average size of 240 bases and a total of 76 Mb (a 38-fold coverage of the genome). These reads were assembled with version 2.1 of the Newbler software yielding 38 large contigs. By comparison with the genome for *Pediococcus acidilactici* 7_4 (accession no. NZ_ACXB00000000), a plausible ordering of these contigs was postulated and primers were designed to join those contigs that were within PCR distance of each other. Thus, we were able to reduce the number of contigs from 38 to 10. By comparing these 10 contigs to *P. acidilactici* DSM 20284 (accession no. NZ_AEEG00000000), we were able to order 9 of them into a single scaffold of 1,953,885 bp with one contig remaining outside this scaffold. The two scaffolds total 1,992,928 bp arranged in 1,967 coding sequences, including 72 RNA genes.

The scaffold was uploaded to the Rapid Annotation using Subsystem Technology (RAST) website (<http://rast.nmpdr.org/rast.cgi>). The G+C content was 42.07%, and there were 270 subsystems represented.

The MA18/5M genome was comparable to the genomes of *Pediococcus pentosaceus* ATCC 25745 (accession no. NC_008525), *P. acidilactici* DSM 20284, and *P. acidilactici* 7_4, but several unique features were identified in the MA18/5M genome. Among the unique aspects was a clustered regularly interspaced short palindromic repeat (CRISPR) located on contig 5 (AGKB01000005.1) and reviewed in reference 6. There were 15 near-identical repeats of 36 nucleotides interspaced with 14 unique spacers of 30 nucleotides each. However, despite the pres-

ence of the CRISPR, 15 genes encoding phage proteins and phage transcriptional regulators were identified. Attempts were made to induce the phage to lyse the bacteria by incubation with mitomycin C as described in the work of Caldwell et al. (1), but all attempts failed (data not shown). There were no open reading frames encoding pediocins, a well-described family of bacteriocins frequently observed in pediococci (5).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [AGKB00000000](https://doi.org/10.1093/nar/gkq000). The version described in this paper is the first version, AGKB01000000.

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REFERENCES

- Caldwell SL, McMahon DJ, Oberg CJ, Broadbent JR. 1999. Induction and characterization of *Pediococcus acidilactici* temperate bacteriophage. *Syst. Appl. Microbiol.* 22:514–519.
- Castex M, Lemaire P, Wabete N, Chim L. 2010. Effect of probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress of *Litopenaeus stylirostris* under *Vibrio nigripulchritudo* challenge. *Fish Shellfish Immunol.* 28:622–631.
- Di Giancamillo A, Vitari F, Bosi G, Savonni G, Domeneghini C. 2010. The chemical code of porcine enteric neurons and the number of enteric glial cells are altered by dietary probiotics. *Neurogastroenterol. Motil.* 22:e271–e278.
- Lessard M, et al. 2009. Administration of *Pediococcus acidilactici* or *Saccharomyces cerevisiae boulardii* modulates development of porcine mucosal immunity and reduces intestinal bacterial translocation after *Escherichia coli* challenge. *J. Anim. Sci.* 87:922–934.
- Rodriguez JM, Martinez MI, Kok J. 2002. Pediocin PA-1, a wide-spectrum bacteriocin from lactic acid bacteria. *Crit. Rev. Food Sci. Nutr.* 42:91–121.
- Sorek R, Kunin V, Hugenholtz P. 2008. CRISPR—a widespread system that provides acquired resistance against phages in bacteria and archaea. *Nat. Rev. Microbiol.* 6:181–186.

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