





Genome Sequence of *Bifidobacterium breve* INIA P734 (CECT 8178), a Strain Isolated from Human Breast Milk

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ABSTRACT The draft genome sequence of *Bifidobacterium breve* INIA P734, a strain shared by mother and child, is reported. It consists of 50 contigs, with 2,391,925 bp, 2,099 genes, and a G+C content of 58.8%. The genome analysis revealed the absence of antibiotic resistance and pathogenicity-related genes.

Isolation of bifidobacteria and lactobacilli shared by mother and child as a source of probiotics has raised considerable interest in the last years (1, 2). *Bifidobacterium breve* is one of the bacteria most frequently isolated from human milk and the infant gut, particularly in breastfed infants (3–5).

Breast milk and infant fecal samples were plated on reinforced clostridial medium (RCM) agar with 0.3 g/liter of methyl blue and incubated anaerobically for 72 h at 37°C for differential isolation of bifidobacteria. Isolates were identified by 16S rRNA gene sequence comparison, and discrimination of bifidobacterial strains within mother-child pairs was established by pulsed-field gel electrophoresis (PFGE) typing (2). *B. breve* INIA P734 (CECT 8178) was isolated from human milk and corresponds to a breast milk-infant feces pair with interesting probiotic (2) and technological (6) properties. *B. breve* is among the species listed as safe by the European Food Safety Authority (EFSA) for the Qualified Presumption of Safety (QPS) status, and that could underwrite its industrial applications for food supplements or fermented products. Therefore, in order to get a deeper knowledge of the probiotic potential and safety properties of this strain, whole-genome sequencing was undertaken. Culturing for genomic DNA (gDNA) isolation, gDNA extraction, and library preparation was performed by GenProbio srl (Parma, Italy), following the methodology described by Lugli et al. (7). Briefly, cells were inoculated in de Man-Rogosa-Sharpe (MRS) medium (Scharlau Chemie) supplemented with 0.05% (wt/vol) L-cysteine hydrochloride and incubated at 37°C in an anaerobic atmosphere. Cells from 10 ml of an overnight culture were harvested by centrifugation at 6,000 rpm for 8 min, and the obtained cell pellet was used for DNA extraction using the GenElute bacterial genomic DNA kit (Sigma-Aldrich) following the manufacturer's guidelines. A genome library was generated using the TruSeq Nano DNA kit. The genome sequences were retrieved using an Illumina MiSeq platform with 2 × 250-bp paired-end reads. A total of 2 × 734,895 raw reads comprising 368,000,944 bp were obtained. Reads, quality controlled with FastQC, were assembled with the software MIRA v4.0.2 (8), and evaluation of the final assembly was done with QCAST v4.3 (9) and CheckM v1.0.7 (10) (with the options lineage_wf and reduced_tree) prior to annotation with Prokka v1.12 (11) (with the options compliant, addgenes, and rfam) and Rapid Annotation using Subsystems Technology (RAST) v2.0 (12) (with the parameters Genetic code 11, Annotation scheme "RASTtk," and the option of Automatically fix errors). The genome was screened with the following tools recommended by EFSA (13) to investigate the relevant genes involved in food safety:

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(i) Resistance Gene Identifier (RGI) of the Comprehensive Antibiotic Resistance Database (CARD) (14) (default parameters) to predict the genes involved in antimicrobial resistance; (ii) PathogenFinder (15) to study bacterial pathogenicity, selecting “All” as the model and “Assembled/Genome Contigs*” as the sequencing platform; and (iii) the BAGEL4 Web server (16) (default parameters) to predict the genes involved in the synthesis of bacteriocins and ribosomally synthesized and posttranslationally modified peptides (RiPPs). The draft achieved comprises 50 contigs with 2,391,925 bp and a G+C content of 58.8%. The largest contig is 241,418 bp, and the N_{50} value is 217,975 bp, with a completeness of 100% and a contamination level of 0.42% (according to the CheckM results). The annotation yielded a total of 2,099 genes (2,031 proteins, 55 tRNAs, 2 rRNAs, and 11 other RNAs). Antibiotic resistance genes and pathogenic and bacteriocin-encoding genes were not found with the above-mentioned tools at the time of analysis, and accordingly, no antimicrobial resistance was detected phenotypically based on the procedures and cutoff values of the EFSA guidance (13).

Data availability. The genome sequence and annotation of *Bifidobacterium breve* INIA P734 have been deposited in the ENA under accession no. CABFNK01000000 (study accession no. PRJEB32824, BioSample accession no. SAMEA5675151, experiment accession no. ERX4268605, and run accession no. ERR4321747). The version described in this paper is the first version.

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REFERENCES

- Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, Rodríguez JM. 2013. The human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res* 69:1–10. <https://doi.org/10.1016/j.phrs.2012.09.001>.
- Peirotn A, Arqués JL, Medina M, Rodríguez-Minguez E. 2018. Bifidobacterial strains shared by mother and child as source of probiotics. *Benef Microbes* 9:231–238. <https://doi.org/10.3920/BM2017.0133>.
- Gueimonde M, Laitinen K, Salminen S, Isolauri E. 2007. Breast milk: a source of bifidobacteria for infant gut development and maturation? *Neonatology* 92:64–66. <https://doi.org/10.1159/000100088>.
- Martín R, Jiménez E, Heilig H, Fernández L, Marín ML, Zoetendal EG, Rodríguez JM. 2009. Isolation of bifidobacteria from breast milk and assessment of the bifidobacterial population by PCR-denaturing gradient gel electrophoresis and quantitative real-time PCR. *Appl Environ Microbiol* 75:965–969. <https://doi.org/10.1128/AEM.02063-08>.
- Soto A, Martín V, Jiménez E, Mader I, Rodríguez JM, Fernández L. 2014. Lactobacilli and bifidobacteria in human breast milk: influence of antibiotic therapy and other host and clinical factors. *J Pediatr Gastroenterol Nutr* 59:78–88. <https://doi.org/10.1097/MPG.0000000000000347>.
- Peirotn A, Gaya P, Arqués JL, Medina M, Rodríguez E. 2019. Technological properties of bifidobacterial strains shared by mother and child. *Biomed Res Int* 2019:9814623. <https://doi.org/10.1155/2019/9814623>.
- Lugli GA, Duranti S, Albert K, Mancabelli L, Napoli S, Viappiani A, Anzalone R, Longhi G, Milani C, Turroni F, Alessandri G, Sela DA, van Sinderen D, Ventura M. 2019. Unveiling genomic diversity among members of the species *Bifidobacterium pseudolongum*, a widely distributed gut commensal of the animal kingdom. *Appl Environ Microbiol* 85:e03065-18. <https://doi.org/10.1128/AEM.03065-18>.
- Chevreaux B, Wetter T, Suhai S. 1999. Genome sequence assembly using trace signals and additional sequence information, p 45–56. *In* Computer science and biology: proceedings of the German Conference on Bioinformatics, GCB '99. GCB, Hannover, Germany.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Rychen G, Azimonti G, Bampidis V, de Lourdes Bastos M, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R, Galobart J. 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA J* 16:e05206. <https://doi.org/10.2903/j.efsa.2018.5206>.
- Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FSL, Wright GD, McArthur AG. 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res* 45:D566–D573. <https://doi.org/10.1093/nar/gkw1004>.
- Cosentino S, Voldby Larsen M, Møller Aarestrup F, Lund O. 2013. PathogenFinder—distinguishing friend from foe using bacterial whole genome sequence data. *PLoS One* 8:e77302. <https://doi.org/10.1371/journal.pone.0077302>.
- Van Heel AJ, De Jong A, Montalbán-López M, Kok J, Kuipers OP. 2013. BAGEL3: automated identification of genes encoding bacteriocins and (non-)bacteriocidal posttranslationally modified peptides. *Nucleic Acids Res* 41:W448–W453. <https://doi.org/10.1093/nar/gkt391>.