## **GENOME ANNOUNCEMENTS**

## Complete Genome Sequence of *Bifidobacterium animalis* subsp. *lactis* BLC1

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Bifidobacterium animalis subsp. lactis BLC1 is a probiotic bacterium that is widely exploited by food industries as the active ingredient of various functional foods. Here we report the complete genome sequence of *B.* animalis subsp. lactis BLC1, which is expected to provide insights into the biology of this health-promoting microorganism and improve our understanding of its phylogenetic relatedness with other members of the *B.* animalis subsp. lactis taxon.

Specific strains belonging to the genus *Bifidobacterium* are extensively exploited by food industries due to their perceived role as health-promoting or probiotic bacteria (10). Genome sequencing and the subsequent functional analysis, as specifically applied to this group of microorganisms and also termed probiogenomics (12), are an important discovery approach to reveal how they adapt to their host and to investigate their mechanisms of probiotic action.

Here, we described the fully decoded genome sequence of the probiotic *Bifidobacterium animalis* subsp. *lactis* BLC1. This strain has been commercially available for more than 7 years (Sacco, Cadorago, Italy), and it is added to several functional foods but it is also present in nutraceuticals and pharmaceutical applications (Sacco, Cadorago, Italy). The complete genome sequence of strain BLC1 was determined using cells from the Sacco bacterial culture collection.

A long-tag paired-end library was constructed from chromosomal DNA and submitted to genome sequencing using a Roche genome sequencer FLX titanium. The generated sequences represented a 30-fold coverage of the genome and were initially assembled into 20 contigs. In order to increase the fold coverage and to improve the quality of the assembly, we employed an additional run of DNA sequencing using Illumina technology. All the generated contigs were assembled using the hybrid assembly strategy based on MIRA software and aligned using the genome of *B. animalis* subsp. *lactis* BB12 (4) as the template. Low-quality DNA regions were verified by Sanger sequencing of PCR products.

The B. animalis subsp. lactis BLC1 genome is composed of

one circular chromosome of 1,943,990 bp displaying a GC content of 60.5%, which is similar to that of other bifidobacterial genomes (11). The BLC1 genome carries 1,560 open reading frames (ORFs), and it possesses 52 tRNA operons and 4 rRNA operons, and this overall genome structure is very similar to that identified in other *B. animalis* subsp. *lactis* chromosomes (1, 4, 9).

The genome structure of *B. animalis* subsp. *lactis* BLC1 is highly syntenic with that of the recently sequenced genome of *B. animalis* subsp. *lactis* BB12 with an average nucleotide identity of 99.9% across these two genomes as determined by the use of a Stretcher alignment (8). The very close phylogenetic relatedness between these two strains was also confirmed by the absence of any of the discriminative single-nucleotide polymorphisms that have been recently described as a valid reference database for analyzing the genomic variability within the *B. animalis* subsp. *lactis* taxon (1).

Clusters of regularly interspersed short palindromic repeat (CRISPR) loci (2, 5–7), which include DNA repeats and the *cas* genes (CRISPR-associated genes) (2), have been shown to be highly variable at the intraspecific level in bifidobacteria (3, 10, 12). Noticeably, in *B. animalis* subsp. *lactis* BLC1, the typical 36-bp CRISPR is identical to those present in the genome of *B. animalis* subsp. *lactis* BB12. All together, the results of these analyses indicate a genomically monomorphic subspecies and show that strains BLC1 to BB12 have a very close isogenic nature.

Nucleotide sequence accession number. The genome sequence of *B. animalis* subsp. *lactis* BLC1 was deposited in GenBank under accession number CP003039.

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## REFERENCES

- Barrangou, R., et al. 2009. Comparison of the complete genome sequences of *Bifidobacterium animalis* subsp. *lactis* DSM 10140 and Bl-04. J. Bacteriol. 191:4144–4151.
- Barrangou, R., et al. 2007. CRISPR provides acquired resistance against viruses in prokaryotes. Science 315:1709–1712.
- Bottacini, F., et al. 2010. Comparative genomics of the genus *Bifidobacte-rium*. Microbiology 156:3243–3254.
- Garrigues, C., E. Johansen, and M. B. Pedersen. 2010. Complete genome sequence of *Bifidobacterium animalis* subsp. *lactis* BB-12, a widely consumed probiotic strain. J. Bacteriol. 192:2467–2468.
- Jansen, R., J. D. Embden, W. Gaastra, and L. M. Schouls. 2002. Identification of genes that are associated with DNA repeats in prokaryotes. Mol. Microbiol. 43:1565–1575.
- Makarova, K. S., N. V. Grishin, S. A. Shabalina, Y. I. Wolf, and E. V. Koonin. 2006. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional

- Mojica, F. J., C. Diez-Villasenor, E. Soria, and G. Juez. 2000. Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. Mol. Microbiol. 36:244–246.
- Rice, P., I. Longden, and A. Bleasby. 2000. EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet. 16:276–277.
- Sun, Z., et al. 2010. Complete genome sequence of probiotic *Bifidobacterium* animalis subsp. lactis strain V9. J. Bacteriol. 192:4080–4081.
- Turroni, F., D. van Sinderen, and M. Ventura. 2011. Genomics and ecological overview of the genus *Bifidobacterium*. Int. J. Food Microbiol. 149:37–44.
- Ventura, M., C. Canchaya, A. Tauch, G. Chandra, K. Chater, G. F. Fitzgerald, and D. van Sinderen. 2007. Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol. Mol. Biol. Rev. 71: 495–548.
- Ventura, M., et al. 2009. Genome-scale analyses of health-promoting bacteria: probiogenomics. Nature Microbiol. Rev. 7:61–71.