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Complete Genome Sequence of *Bifidobacterium animalis* subsp. *lactis* BB-12, a Widely Consumed Probiotic Strain▽

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***Bifidobacterium animalis* subsp. *lactis* BB-12 is a commercially available probiotic strain used throughout the world in a variety of functional foods and dietary supplements. The benefits of BB-12 have been documented in a number of independent clinical trials. Determination of the complete genome sequence reveals a single circular chromosome of 1,942,198 bp with 1,642 predicted protein-encoding genes, 4 rRNA operons, and 52 tRNA genes. Knowledge of this sequence will lead to insight into the specific features which give this strain its probiotic properties.**

The consumption of bifid bacteria to restore a healthy gut microflora was first suggested in 1906 (8). Since then, the use of probiotic bacteria to confer health benefits has developed into a global industry in which probiotic bacteria are delivered to consumers in functional foods, infant formulas, and dietary supplements. A number of species, primarily of the genera *Bifidobacterium* and *Lactobacillus*, are employed. It is generally believed that the ability of a strain to provide a health benefit is a strain-specific property (see, for example, reference 6). Research on strains with documented probiotic capabilities is therefore of particular scientific interest.

Bifidobacterium animalis subsp. *lactis* strain BB-12 has been commercially available for more than 25 years and is the subject of numerous independent clinical trials. We are aware of more than 200 scientific publications involving BB-12 (see, for example, reference 5), making this one of the most thoroughly studied probiotic strains available. We have determined the complete genome sequence of BB-12 by using cells from the inoculation material employed for commercial production of this strain.

Genomic DNA was shotgun cloned into plasmid and cosmid vectors and sequenced by Integrated Genomics (Chicago, IL) using Sanger methodology. This resulted in 11-fold coverage of the genome and, initially, 56 contigs. Initial alignment of the contigs was done by comparison to the published genome sequence of *B. longum* NCC2705 (7). Even though NCC2705 shows little homology to BB-12 at the DNA sequence level, several large clusters of genes were found to have the same organization, allowing alignment and closing of several gaps. The remaining gaps were closed by combinatorial PCR using 70 primers designed from sequences at the ends of the original contigs and by additional DNA sequencing following primer walking. This approach resulted in a single circular contig containing 1,942,198 bp. No plasmids are present in BB-12.

An optical map of the BB-12 chromosome digested by NotI was produced by OpGen (Madison, WI) and analyzed using the OpGen MapSolver package. There are 193 NotI sites in the genome sequence, and the optical map confirmed the correctness of the assembly. The overall chromosomal structure is the same as that determined for the type strain DSM 10140 (1), whereas strain AD011 (4) has apparently undergone a number of genome rearrangements, yielding several differences in the *in silico*-predicted locations of NotI sites. Alternatively, the differences in AD011 may be due to sequence misassembly, possibly because of a failure to detect two rRNA operons: the AD011 genome sequence contains only two rRNA operons, whereas BB-12 and DSM 10140 contain four.

Gene finding and annotation of the genome were done by Integrated Genomics using the ERGO genome analysis suite. This led to the identification of 1,642 potential open reading frames, of which approximately 70% could be assigned a putative function. In addition, 4 rRNA operons and 52 tRNA genes were identified. The sequence was used to design whole-genome microarrays. This microarray platform has been used to analyze and improve biomass production of BB-12 through transcriptomics, to compare *B. animalis* subsp. *animalis* and *B. animalis* subsp. *lactis* strains by comparative genome hybridization (3), and to gain further insight into the general physiology and potential mode of action of BB-12.

The benefits of using genome sequence information to characterize probiotic bacteria has been described previously (2, 3), and it is anticipated that the knowledge of the complete genome sequence of BB-12 will lead to further gene-based understanding of the probiotic properties of this strain.

Nucleotide sequence accession number. The genome sequence of *B. animalis* subsp. *lactis* BB-12 is deposited in GenBank under accession number CP001853.

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BB-12 is a registered trademark of Chr Hansen A/S.

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