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Complete Genome Sequence of the Probiotic *Lactobacillus casei* Strain BL23[∇]

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The entire genome of *Lactobacillus casei* BL23, a strain with probiotic properties, has been sequenced. The genomes of BL23 and the industrially used probiotic strain Shirota YIT 9029 (Yakult) seem to be very similar.

The genome of *Lactobacillus casei* BL23 was sequenced using a combination of shotgun sequencing (performed by Cognetics, Meylan, France) and 454 pyrosequencing (DNA sequencing was performed by Genoscope, Evry, France). Based on 19,300 reads of the shotgun library, draft assemblies were compiled with Consed, providing 90 contigs and 4.5-fold coverage. The 400,000 pyrosequencing reads were assembled into 132 contigs. The large number of contigs owes to numerous repeated sequences, most of which were already correctly positioned in the shotgun approach. A combined assembly of the two data sets therefore provided only 13 contigs with 34-fold coverage. Twelve gaps were closed by PCR using combinations of 26 primers corresponding to the contig extremities. The last gap was located at the insertion site of a prophage (lclb23-2), and the pyrosequencing data allowed two different assemblies corresponding to the integrated and circularized phages. For each assembly, about 30 different reads had been obtained. Apparently, only a few cells contain a mobilized prophage, because only a single pyrosequencing read corresponded to the BL23 genome without this phage. Excised lclb23-2 probably rapidly propagates, explaining why reads corresponding to the circularized prophage were more abundant.

The BL23 genome is composed of a single circular chromosome (3,079,196 bp) with an overall G+C content of 46.34%. Coding sequence (CDS) prediction and annotation were carried out with AGMIAL (2) and provided 3,044 CDSs covering 84% of the genome. BL23 also harbors 5 rRNA operons and 60 tRNAs. The origin of replication was identified based on homology to the *L. casei* neotype strain ATCC 334 (5, 7). Most

genes of BL23 are present on the leading strand. The replication terminus is located almost diametrically opposite to the origin of replication and is accompanied by a sharp transition in the GC skew.

Almost all genes present in BL23 and ATCC 334 exhibit nearly (99%) identical sequences, and the synteny is widely conserved in the two chromosomes, with only a few extended homologous regions placed at different locations. Interestingly, a published 15-kb sequence of the probiotic Shirota strain (10) differs from the region covered by the genes LCABL_22200 to LCABL_22350 of BL23 at only two positions. The BL23 CDSs are also nearly (98%) identical to their homologues in *Lactobacillus paracasei*. The BL23 genome is almost 0.2 Mb bigger than that of ATCC 334 (7), which nevertheless contains numerous DNA regions absent from BL23. The core genome common to both strains is about 2.38 Mb (77% of the BL23 genome). A significant fraction of the accessory genome owes to prophage insertions and insertion elements. Other regions present in only one strain, such as the 25-kb region extending from genes LCABL_28260 to LCABL_28470 in BL23, are often related to carbohydrate utilization, which may reflect the different environments to which the two *L. casei* strains adapted (3). Strain ATCC 334 is a cheese isolate (7), while BL23 was obtained in trying to cure *L. casei* ATCC 393 (reclassified as *Lactobacillus zaei*) (5) of a plasmid. The isolated plasmid-free BL23 exhibits probiotic properties (6, 9) and is easily transformable and widely used for physiological, genetic, and biochemical studies (8). However, there is evidence that ATCC 393 is not the ancestral strain for BL23 (1, 4).

Nucleotide sequence accession number. The genome sequence was deposited at EMBL with the accession number FM177140.

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