Complete Genome Sequence of *Lactobacillus casei* Zhang, a New Probiotic Strain Isolated from Traditional Homemade Koumiss in Inner Mongolia, China⁷

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Lactobacillus casei Zhang is a new probiotic bacterium isolated from koumiss collected in Inner Mongolia, China. Here, we report the main genome features of L. casei Zhang and the identification of several predicted proteins implicated in interactions with the host.

Koumiss, a traditional drink made from mare's milk by nomadic peoples in China and Mongolia, is believed to be beneficial in the cure of digestive diseases and a wide range of chronic diseases, including tuberculosis, bronchitis, and anemia (3). *Lactobacillus casei* Zhang is a novel probiotic strain identified by screening of lactic acid bacteria isolated from koumiss samples collected in Inner Mongolia, China, and exhibits high-level resistance to acid and bile stresses, as well as antibacterial, antioxidative, and immunomodulatory properties (6, 7, 11).

A whole-genome shotgun strategy was used for sequencing of the genome of *L. casei* Zhang. pUC18 plasmid libraries with insertions of 1.5 to 2.5 kb and 4 to 6 kb were constructed (8). Gaps were closed by sequencing of PCR products. Base calling and sequence assembly were carried out using the Phred/Phrap/Consed software package (http://www.phrap.org/), and reads giving a total of 6.2-fold coverage were assembled with an error rate of <0.0001. Gene prediction and annotation were performed as described previously (10).

The complete genome of *L. casei* Zhang consists of a 2,861,848-bp circular chromosome and a 36-kb plasmid. The average G+C content of the chromosome is 46.5%, while the plasmid has a lower G+C content (10). The *L. casei* Zhang genome contains 2,804 predicted coding sequences (CDSs), five rRNA operons, and 59 tRNAs. No functional prophages were identified, except for the previously described prophage

remnant (9). Genes for 41 transposases were found in the genome, and this number was much lower than (only about 30%) those of transposase genes in *L. casei* ATCC 334 and BL23 (1, 4), suggesting that insertion element (IS)-mediated genome diversification was less frequent in *L. casei* Zhang.

Comparative genome analysis revealed that the number of phosphotransferase system (PTS)-related proteins varied significantly in L. casei strains. Almost twice as many PTS components were found in L. casei Zhang and BL23 as in L. casei ATCC 334. In contrast to L. casei ATCC 334, L. casei Zhang was found to have 33 PTS components consisting of 11 complete substrate-specific enzyme II (EII) complexes encoded by six genomic islands. The G+C contents of the six islands ranged from 41 to 47%, similar to the average G+C content of the L. casei Zhang genome. In addition, most of the EII components in L. casei Zhang (81 of 96) were conserved in L. casei BL23, suggesting that a large-scale loss of PTSs occurred in L. casei ATCC 334 during its evolution. Conspicuous redundancy of chromosome-encoded PTSs in L. casei Zhang may offer benefits in the transport and use of a large panel of carbon sources.

Genes encoding five putative mucus-binding proteins (LCAZH_0407, LCAZH_2292, LCAZH_2478, LCAZH_2398, and LCAZH_1427) and a cluster of genes encoding bacteriocin biosynthetic proteins (LCAZH_2341 to LCAZH_2348) nearly identical to those in *L. casei* ATCC 334 and BL23 were identified in *L. casei* Zhang and may provide this bacterium with some competitive advantages in the gastrointestinal environment (2, 5).

In conclusion, the comparative analysis revealed the flexibility of *L. casei* Zhang in sugar utilization. In addition, some possible hints for its interactions with the host were identified. This genome sequence will be the basis for systematic studies into the mechanism for the probiotic properties of *L. casei* Zhang.

Nucleotide sequence accession number. The complete nucleotide sequence of the *L. casei* Zhang chromosome was de-

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posited in GenBank under the accession number CP001084. More detailed annotations are available from the Chinese Probiotics Network (http://www.bio149.cn/).

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