

# Complete Genome Sequence of *Lactobacillus kefiranofaciens* ZW3<sup>∇</sup>

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***Lactobacillus kefiranofaciens* ZW3 was isolated in Tibet, China, from kefir grain, a traditional dairy product that is known to provide many health benefits to humans. Here, we present the genome features of *L. kefiranofaciens* ZW3 and the identification of a gene cluster related to the synthesis of exopolysaccharide, an important constituent of the Tibetan kefir.**

Kefir is a viscous, slightly carbonated dairy beverage (5) which provides many health benefits (10, 14) and uses kefir grain as the starter culture. The main beneficial effects are believed to have derived from bacterially produced exopolysaccharides (EPS) (1, 8, 13). *Lactobacillus kefiranofaciens* is one of the major microbial constituents of kefir grain that forms highly viscous colonies (12, 18). *L. kefiranofaciens* ZW3 is a novel strain isolated from kefir grain collected in Tibet, China. Among many outstanding characteristics, it exhibits a strong ability to produce EPS (16). However, few studies have reported the genomic information for *L. kefiranofaciens*, let alone the complete genome. Here, we report the complete genome sequences of *L. kefiranofaciens* ZW3, representing the first genome of *L. kefiranofaciens*.

Whole-genome sequencing was performed using a combination of 454 sequencing technology (517,476 reads totaling ~96.732 Mb; ~45-fold coverage) and GA II× Solexa high-throughput sequencing (9,866,448 reads totaling ~986 Mb; ~460-fold coverage). Contigs were generated using Newbler Assembler software (454 Life Sciences Corporation) and assembled into scaffolds using paired-end reads. Physical gaps, repeats, and assembly ambiguities were corrected by multiplex PCR and ABI sequencing. Genome finishing was carried out using the Phred/Phrap/Consed software package (3, 4, 6). Protein-coding genes were predicted by Glimmer and Genemark (2). Artemis was used for final verification of the annotation results (15).

The complete genome of ZW3 contains a single, circular chromosome of 2,113,023 bp and two plasmids (pWW1 [194,769 bp] and pWW2 [46,296 bp]). The overall GC content of the chromosome is 37.70%, whereas the two plasmids have GC contents of 33.98% and 36.02%, respectively. This genome size is smaller than that of *Lactobacillus plantarum* JDM1 (3.197 Mb) (20), *Lactobacillus casei* BL23 (3,079 M) (9), *Lactobacillus rhamnosus* ATCC 53103 (3,005 M) (11), and *Lactobacillus casei* Zhang (2,861 M) (19) but slightly larger than the complete genomes of *Lactobacillus johnsonii* FI9785 (1,756 M) (17). The chromosome contains 1,908 predicted protein-encoding sequences (CDSs), 60 tRNA-encoding genes, and 4 rRNA-encoding genes. Plasmids

pWW1 and pWW2 carry 199 and 55 predicted protein-encoding sequences, respectively.

One of the most significant features of ZW3 is the ability to produce high-yield EPS. The ZW3 genome carries a 14.4-kb EPS gene cluster (WANG\_1283 to WANG\_1299), which contains 17 EPS-related genes. These genes showed high similarity to those which are involved in the regulation, polymerization, chain length determination, and export of the EPS. Twelve of these genes have some homologies with other *Lactobacillus* species, including *L. helveticus* and *L. amylovorus*, etc. The remaining 5 genes in the EPS gene clusters are uniquely present in the ZW3 and regarded as the key enzymes to determine the formation of unique EPS (7).

In conclusion, the genome sequence provides new avenues to further explore gene-based functional and probiotic mechanisms of ZW3. In addition, comparative genomics analysis and functional genomics analysis could also be carried out to trace the origin and evolution of this bacterium.

**Nucleotide sequence accession numbers.** The *Lactobacillus kefiranofaciens* ZW3 chromosome and plasmid pWW1 and pWW2 sequences have been deposited in GenBank under accession numbers CP002764, CP002765, and CP002766.

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