

## Draft Genome Sequence of Lactobacillus casei W56

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We announce the draft genome sequence of *Lactobacillus casei* W56 in one contig. This strain shows immunomodulatory and probiotic properties. The strain is also an ingredient of commercially available probiotic products.

**B** acteria with potential probiotic abilities affecting the human immune system are increasingly gaining importance for human wellbeing in today's life. *In vitro* studies have shown that *Lactobacillus casei* strain W56 (Winclove Bioindustries, Amsterdam, Netherlands) has immunomodulatory abilities to human dendritic cells and a thymus and activation-regulated chemokine (TARC)-decreasing activity in KM-H2 cells (Kepert) (I. Kepert, J. Fonseca, K. Hochwind, A. Hartmann, P. Schmitt-Kopplin, and S. Krauss-Etschmann, unpublished data). Moreover, there are other probiotic characteristics described, like a significant increase of the production of the proinflammatory cytokine tumor necrosis factor alpha (TNF- $\alpha$ ) and a reduction of interleukin 6 (IL-6) in peripheral blood mononuclear cells (15). Therefore, a complete genome sequence would provide us with detailed information about the genetic basis of possible probiotic mechanisms, which are not understood so far.

The strain was grown under microaerobic conditions in MRS broth (Applichem, Darmstadt, Germany) at 37°C. Genomic DNA extraction was performed with a FastDNA Spin kit for soil (MP Biomedicals, Illkirch, France). For genome sequencing, we used Roche 454 GS (FLX Titanium) pyrosequencing (320,156 sequenced reads containing  $\sim$ 111.5 Mb;  $\sim$ 34-fold coverage of the genome). We performed a comparative assembly using AMOScmp (13) based on the genome of the closely related strain L. casei BL23 (12) as a reference. The assembly generated four contigs. Two of them correspond to the chromosome, and the other two match to an extrachromosomal plasmid. The remaining gaps were closed by PCR, and Sanger sequencing (performed by Sequiserve, Vaterstetten, Germany) resulted in a chromosome size of 3,075,780 bp and a plasmid size of 56,316 bp. Additionally, we confirmed regions in which transposons are located either in the reference or in the sequenced W56 strain by PCR. The G+C contents were 46.3% for the chromosome and 43.7% for the plasmid.

Prediction of coding sequences (CDS) was done using an inhouse workflow that integrates *ab initio* predictions from Glimmer (5), Genemark (11), Prodigal (8), and Critica (3), with homology information derived from a BLAST (2) search against NCBI NR (14). Noncoding RNAs were identified by tRNAscanSE (10), RNAmmer (9), and the Rfam database (6), and functional annotation of CDS was based on interproScan (7) and homology searches against the databases Swiss-Prot (4) and trEMBL (4). The chromosome contains 3,098 coding sequences and 60 genes coding for tRNAs. Ribosomal operons coding for 5S, 16S, and 23S rRNA were present in 5 copies. The sequence of the plasmid has a high similarity to the known plasmid pBDII (1), missing a 1,038bp-long sequence, which is covered mostly by transposases.

Further sequence analysis and knockout mutants of genes of interest will give more information about the mechanism behind the immunomodulatory activity of *L. casei* W56. This can provide

the basis for better understanding of the interactions of probiotic bacteria and the human intestine.

Nucleotide sequence accession numbers. The genome sequence was deposited at EMBL with the accession numbers HE970764 and HE970765.

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