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Genome announcement

# Complete genome sequence of *Lactobacillus helveticus* KLDS1.8701, a probiotic strain producing bacteriocin

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ABSTRACT

This study investigated the functional diversity of *Lactobacillus helveticus* KLDS1.8701 by carrying out a whole-genome sequence analyses of *L. helveticus* KLDS1.8701. *L. helveticus* KLDS1.8701 strain was isolated from traditional sour milk in Sinkiang of China with desirable probiotic properties. Here we report the complete genome sequence of this organism and its genetic basis for adhesion, exopolysaccharides (EPS) production, acid and bile tolerance, bacteriocin production and immune system against bacteriophage.

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*Lactobacillus helveticus* is present in several fermented foods and also is used as a probiotics with many health-promoting properties (Champagne et al., 2010). Being generally recognized as safe (GRAS), strains of this species emerge as an adjunct culture for diverse cheeses with highly efficient proteolytic activity. These strains also produce peptides with a biological function, for instance, peptides possessing an inhibitory activity on the angiotensin converting enzyme (Giraffa, 2014). Clinical trials have demonstrated the effect of *L. helveticus* against diseases such as cancer and intestinal inflammation (Prajapati et al., 2011). The *L. helveticus* KLDS1.8701 strain was originally isolated from traditional sour milk in Sinkiang of China. In a previous *in vitro* gastrointestinal tract tolerance test, it was reported that *L. helveticus* KLDS1.8701 could resist simulated gastric juice, intestinal juice and bile salt, with the number of viable bacteria remained at more than $1 \times 10^6$ CFU/mL after 3 h, indicating that this strain could reach the intestine in a viable state. Lymphocyte proliferation test showed that it could accelerate the proliferation of spleen cells in a dose-dependent manner (Wang et al., 2013). An *in vivo* study using animal model suggests that this strain could regulate the imbalance of gut microbes in diseased mice, and improve the intestinal mucosal immune system by stimulating cytokines and SIgA production, thus exerting a therapeutic effect on diarrhea disease (Wang et al., 2014). These results offer a theoretical basis for the development of drugs and a potential probiotic strain. Today, it has been used in the production of lactein tablets by a pharmaceutical factory in Heilongjiang province. Lactein is normally used in the treatment of indigestion, enteritis, children's diarrhea and other diseases.

To generate genomic insights into the functional diversity of *L. helveticus* KLDS1.8701, we performed the whole-genome sequence of *L. helveticus* KLDS1.8701 using a strategy of Illumina paired-end sequencing technology. Long-insert (5 kb), medial-insert (2.5 kb) and short-insert (500 bp) libraries were constructed for the Illumina Hiseq 2000 platform. A total of 784 M clean data was generated from 3 libraries giving 372-fold coverage of the genome, *De novo* assembly was conducted by SOAPdenovo 2.0 (Luo et al., 2012), which resulted 3 scaffolds with an N50Contig length of 130,432 bp long, there are only 2 gaps between scaffolds, then gaps were filled by sequencing PCR products using an ABI 3730 capillary sequence. Gene annotation was managed by NCBI Prokaryotic Genome annotation Pipeline. CRIPER finder (http://crispr.u-psud.fr/Server/), was used for searching CRISPR/Cas system. Genes potentially involved in the biosynthesis of bacteriocins were identified using BAGEL (http://bagel2.molgenrug.nl/).

The complete genome of *L. helveticus* KLDS1.8701 is composed of a circular chromosome of 2,096,031 bp with a GC content of 37% and a plasmid of 10,600 bp with a GC content of 34%. The chromosome

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**Table 1**
General genome features of *Lactobacillus helveticus* KLDS4.0325.

| Feature | Chromosome | Plasmid |
|---|---|---|
| Size [bp] | 2,096,031 | 10,600 |
| GC content [%] | 36.88% | 34% |
| Predicted genes | 2164 | 13 |
| Protein coding genes (CDSs) | 1763 | 10 |
| Pseudogenes | 327 | 3 |
| rRNA operons | 12 | 0 |
| tRNAs | 61 | 0 |
| ncRNA | 1 | 0 |
| GenBank accession | CP009907.1 | CP009908.1 |

of *L. helveticus* KLDS1.8701 contains 1763 CDSs, 77 RNAs and 327 pseudogenes (Table 1).

We compared the genome of *L. helveticus* KLDS1.8701 obtained in this study with publicly available complete genome sequences of *L. helveticus* strains DPC4571 (GenBank accession no. NC 10080.1) (Callanan et al., 2008), H10 (GenBank accession no. NC 17467.1) (Zhao et al., 2011), R0052 (GenBank accession no. NC 18528.1) (Tompkins et al., 2012), CNRZ32 (GenBank accession no. NC 21744.1) (Broadbent et al., 2013), H9 (GenBank accession no. CP002427) (Chen et al., 2015), MB2-1 (GenBank accession no. CP011386) (Li et al., 2015). *L. helveticus* KLDS1.8701 has more mucus-binding proteins (HUO_04880, HUO_07225, HUO_07230, HUO_07235) than other sequenced *L. helveticus*, indicating that this strain may have superiority to colonize in the intestinal tract and interact with the gut microorganisms as probiotics. In addition, it also has a disrupted gene encoded adhesion (HUO_04755). The KLDS1.8701 genome carries an EPS cluster including 13 genes. HUO_10125 and HUO_10110 encode for biosynthesis regulation, HUO_10120 and HUO_10115 encode for determining the chain length, HUO_10105, HUO_10100, HUO_10095, HUO_10085, HUO_10080, HUO_10075, HUO_10050 encode for glycosyltransferases, HUO_10090 encodes for polymerization event, HUO_10040 encodes for exporting EPS. As compared to other sequenced genomes, HUO_10110, HUO_10090, HUO_10085, HUO_10050, HUO_10040 are unique genes in this cluster, they may endue EPS of *L. helveticus* KLDS1.8701 a distinctive characteristic. We have previously reported the ability of *L. helveticus* KLDS1.8701 to survive simulated gastric juice (pH 2) and Intestinal juice (pH 7.5), 3 g/kg bile salts (Wang et al., 2013). Regarding how genetic elements of *L. helveticus* KLDS1.8701 contributes to acid tolerance, our results showed that *L. helveticus* KLDS1.8701 possesses proton motive force $F_1F_0$ATPase subunits (HUO_00815, HUO_04975, HUO_06740, HUO_08125 to HUO_08160) to actively remove protons from the cytoplasm. Furthermore, $Na^+/H^+$ antiporters (HUO_00630, HUO_06055, HUO_06835, HUO_09320) and $H^+$–$K^+$-exchanging ATPase (HUO_08750) also make contributions to regulate pH of intracellular. Surprisingly, *L. helveticus* KLDS1.8701 displayed a lack of bile salt hydrolases and conjugated bile acid hydrolases genes. There is a gene encoded cyclopropane-fatty-acyl-phospholipid synthase (HUO_05315) in the genome of *L. helveticus* KLDS1.8701 to establish its ability to tolerate bile acid. Detailed analysis of the genome revealed four genes (HUO_02275, HUO_03195, HUO_06670, HUO_07285) encoding for bacteriocin, especially HUO_07285, which can't find its homology, may endow some competitive advantages for bacteria when producing lactein in commercial use, 3CRISPR/Cas loci and the restriction modification system (the Type I, Type II and Type III) were identified in the genome of *L. helveticus* KLDS1.8701, these data suggest that this strain may own a better defense system against bacteriophages, therefore, this strain may be suitable for industrial production.

The complete genome chromosome and plasmid sequence have been deposited in GenBank database with accession number CP009907.1 and CP009908.1, respectively. This strain has been deposited at the China General Microbiological Culture Collection Center (CGMCC No.6639).

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