



Genome announcement

Complete genome sequence of *Lactobacillus plantarum* LZ95, a potential probiotic strain producing bacteriocins and B-group vitamin riboflavin



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ABSTRACT

Lactobacillus plantarum LZ95 is a potential probiotic isolated from newborn infant fecal and it is identified to produce riboflavin with great antimicrobial activity. The complete genome sequence of this strain was reported in the present study. The genome contains a 3,261,418-bp chromosome and two plasmids. Genes, related to the biosynthesis of bacteriocins and riboflavin, were identified. This work will facilitate to reveal the biosynthetic mechanism of bacteriocins and B-group vitamins in lactic acid bacteria and provide evidence for its potential application in food industry.

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Lactobacilli are inherent members in the gastrointestinal tract of human and animals, and several commensal species of such groups have attracted great attention for their probiotic properties and positive impact on the health of hosts (Capozzi et al., 2012; Suo et al., 2012; van Baarlen et al., 2013). *Lactobacillus plantarum* is a highly versatile lactic acid bacteria (LAB) and some of them are marketed as starter cultures or probiotics (Siezen et al., 2010) because of the excellent traits, such as the capacity of producing bacteriocins or B-group vitamins (Capozzi et al., 2011; Masuda et al., 2012). We have previous isolated a *Lactobacillus plantarum* strain ZJ316 from infant fecal which significantly improved pig growth and pork quality, and exhibited antimicrobial activity against various pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* etc. (Suo et al., 2012). The presence of bacteriocin producing gene cluster in ZJ316, identified by complete genome sequencing, could at least partially explain these probiotic properties (Li et al., 2013). LZ95, a novel *Lactobacillus plantarum* strain also isolated from newborn infant fecal, showed comparable antimicrobial activity against the pathogens in our studies. Moreover, *Lactobacillus plantarum* LZ95 was able to produce B-group vitamin riboflavin (B₂), whereas was not produced by ZJ316, showing a greater potential as a probiotic. In order to gain insights about

genetic elements involved in its probiotic properties, we sequenced the entire genome of *Lactobacillus plantarum* LZ95 in this work.

Genomic DNA was isolated using the Qiagen DNA extraction kit and subsequently sequenced with a PacBio RS II platform yielding 47,177 reads with an average length of 10,583 nt. The reads were de novo assembled with HS HGAP Assembly version 2 (Pacific Biosciences), resulting in 3 major contigs with more than 70-fold average coverage. The final contig was checked for circularization and overlapping ends were trimmed. To check the sequence quantity of SMRT (the single molecule real-time), a new 300-bp library for Illumina paired-end was sequenced. Reads were aligned to the reference sequence using the breseq-0.24.rc6 pipeline (Deatherage and Barrick, 2014).

The complete genome sequence of *Lactobacillus plantarum* LZ95 is composed of a circular 3.26 Mb chromosome and two plasmids named as LZ95p1 (48556 bp) and LZ95p2 (12484 bp), with GC contents of 44.6%, 39.0% and 36.4%, respectively (Table 1). There are a total of 3041 genes (2904 protein-coding genes), 16 rRNA operons, 70 tRNAs and 51 pseudo genes found in the chromosome and 58 genes (47 protein-coding genes) and 11 pseudo genes in the plasmids.

15 Genes involved in plantaricin production were identified in the genome of *Lactobacillus plantarum* LZ95, i.e. *plnL* (AD081.01655), *plnK* (AD081.01660), *plnJ* (AD081.01665), *plnM* (AD081.01670), *plnO* (AD081.01680), *plnP* (AD081.01685), *plnA* (AD081.01695), *plnB* (AD081.016100), *plnC* (AD081.016105), *plnD* (AD081.016110), *plnI* (AD081.016115), *plnF* (AD081.016120), *plnE*

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Table 1
Features of *Lactobacillus plantarum* LZ95 genome.

Attributes	Values
Genome size (bp)	3,322,458
GC content (%)	44.4873%
Plasmid	2
rRNAs	16
tRNAs	70
ncRNA	1
Proteins	2951
Predicted genes	3099
Pseudo gene	62

(AD081_016125), *plnG* (AD081_016300) and *plnH* (AD081_016135). It is notable that three different bacteriocin precursor peptides could be encoded: *plnA* encodes class IIc plantaricin A presursor peptide PlnA, *plnE* and *plnF* encode class IIb bacteriocin precursor peptide PlnE and PlnF, *plnJ* and *plnK* encode class IIb bacteriocin precursor peptide PlnJ and PlnK. These results provided more clues to explain the antimicrobial activity of LZ95.

Furthermore, a complete functional *rib* operon, responsible for the riboflavin synthesis, was identified. The *rib* operon contained four genes in LAB, namely *ribH* (6,7-dimethyl-8-ribityllumazine synthase or riboflavin synthase beta subunit, AD081_10085), *ribA* (3,4-dihydroxy-2-butanone 4-phosphate synthase, AD081_10090), *ribB* (riboflavin synthase subunit alpha, AD081_10095) and *ribG* (riboflavin-specific deaminase and reductase, AD081_100100). Some LAB strains, such as *Lactobacillus plantarum* WCFS1, are incapable of producing riboflavin because of the lacking *rib* operon or with incomplete *rib* operon (Burgess et al., 2006; Kleerebezem et al., 2003; LeBlanc et al., 2011). These results provided molecular evidence for the riboflavin production, and thus allowed LZ95 as a potential candidate to prevent B₂ deficiency by *in situ* fortification in food industry. The property of bacteriocin production of the strain could improve the safety and increase the quality of *in situ* fermented products.

Together, the complete genome sequence of *Lactobacillus plantarum* LZ95 will facilitate the biosynthetic mechanism studies of bacteriocins and B-group vitamins of LAB. Future studies are needed to verify the probiotic properties and safety of this strain for its industry application.

Nucleotide sequence accession number

The complete genome sequence of *Lactobacillus plantarum* LZ95 was deposited at GenBank under accession number CP012122–CP012124. This strain has been deposited in China General Microbiological Culture Collection Center under accession number CGMCC No. 11570.

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