Complete genome sequence of *Lactobacillus plantarum* LZ206, a potential probiotic strain with antimicrobial activity against food-borne pathogenic microorganisms

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Abstract:

Lactobacilli strains have been considered as important candidates for manufacturing “natural food”, due to their antimicrobial properties and generally regarded as safe (GRAS) status. *Lactobacillus plantarum* LZ206 is a potential probiotic strain isolated from raw cow milk, with antimicrobial activity against various pathogens, including Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), Gram-negative bacteria (*Escherichia coli* and *Salmonella enterica*), and fungus *Candida albicans*. To better understand molecular base for its antimicrobial activity, entire genome of LZ206 was sequenced. It was revealed that genome of LZ206 contained a circular 3,212,951-bp chromosome, two circular plasmids and one predicted linear plasmid. A plantaricin gene cluster, which is responsible for bacteriocins biosynthesis and could be associated with its broad-spectrum antimicrobial activity, was identified based on comparative genomic analysis. Whole genome sequencing of *L. plantarum* LZ206 might facilitate its applications to protect food products from pathogens’ contamination in the dairy industry.

Keywords: *Lactobacillus plantarum* LZ206; Complete genome sequence; antimicrobial activity; bacteriocins; food-borne pathogenic microorganisms

Contamination of food products (e.g. milk and dairy products) during food transport, processing or storage by microorganisms that are pathogenic to humans, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enterica*, often leads to severe food-borne disease (Field *et al.*, 2016; Fox *et al.*, 2009). Varieties of chemical compounds have been applied as antimicrobials to inhibit pathogens in food products. However, consumers prefer to have “natural food” for the concern of health in recent decades. To eliminate or reduce chemical additives, scientific researches and food industry focus on natural antimicrobials for processing of food products. They figured out that microbial molecules and/or bacterial strains that are able to produce such compounds could be suitable for the “natural purpose” as potential antimicrobials and preservatives. Lactic acid bacteria (LAB) are considered as one of the greatest candidates for manufacturing “natural food”, safe and healthy (Mangiapane *et al.*, 2015).

Lactic acid bacteria (LAB), especially strains belonging to genus *Lactobacillus* such as *L. plantarum* strains, are widely used as probiotics and functional starter cultures in fermented food products, due to their strain specific health properties and the generally regarded as safe (GRAS) status (Giraffa *et al.*, 2010). Several *L. plantarum* strains have been found to produce different antimicrobial compounds and exhibit antagonistic activity against pathogenic and spoilage microorganisms (Song *et al.*, 2014; Suo *et al.*, 2012; Zhu *et al.*, 2014). One group of these compounds, the bacteriocins, is the ribosomally synthesized antimicrobial peptides and works against closely related species (Klaenhammer, 1993). In the food industry, the bacteriocin nisin, produced by *Lactococcus lactis* subsp. *lactis*, has already been used in more than 50 countries as an antagonistic additive to reduce the addition of chemical preservatives (Field *et al.*, 2016). Bacteriocins produced by *L. plantarum* are named as plantaricins, most of which, such as PlnA and the two-peptide bacteriocins PlnJ/K, PlnE/F and plantaricin NC8, belong to class II. Some of these plantaricins have antibacterial activity against both Gram-positive and Gram-negative bacteria (e.g. *Salmonella enterica* and *Escherichia coli*) (Jiang *et al.*, 2016; Pal and Srivastava, 2014), indicating a great potential of *L. plantarum* as antimicrobials.

Although nisin has already been applied to food products (e.g. dairy products) to inhibit pathogens' contamination, it is limited to Gram positive pathogens (mainly *Listeria monocytogenes*), and also, addition of nisin would increase the cost. *L. plantarum* strains have been utilized for *in situ* fortification in food industry,

especially in the dairy industry. Thus, it could be a good idea to identify novel *L. plantarum* strains with antimicrobial activity, and use strains for both *in situ* fortification and protection the dairy products from pathogens.

In the present study, we isolated a novel *L. plantarum* strain from raw cow milk named as LZ206. It displayed a similarity of $\geq 99\%$ to lactobacilli strains deposited in NCBI based on the 16s rRNA analysis. Fig.1 showed a distinct branch of LZ206 with *L. plantarum* strains in the phylogenetic tree and *L. plantarum* ZJ316 was the closest evolutionary relative of LZ206, therefore this strain is preliminarily recognized as *L. plantarum* ssp. Table 1 showed that supernatant (pH 3.6) of *L. plantarum* LZ206 had strong inhibitory effects on Gram-positive bacteria, including pathogenic bacteria *Staphylococcus aureus* and *Listeria monocytogenes*, which could contaminate dairy products and cause severe human diseases such as infective endocarditis, gastroenteritis, maternofetal infections and meningoencephalitis *etc.* (Field *et al.*, 2016). Different from most bacteriocins, *L. plantarum* LZ206 culture supernatant significantly inhibited two major food-borne Gram-negative pathogens *Escherichia coli* and *Salmonella enterica*, which is listed as a serious hazard and moderate hazard of foodborne pathogen by WHO (Schlundt *et al.*, 2004). Moreover, it showed inhibitory activity against the fungus *Candida albicans*. To exclude the possibility that the antimicrobial activity of *L. plantarum* LZ206 was due to low pH, we observed its antimicrobial activity at pH 5.0. The supernatant (pH 5.0) still exhibited an inhibitory activity against all the tested pathogens (data not shown). Together, *L. plantarum* LZ206 showed inhibitory activity against varieties of Gram positive and Gram negative bacteria, and fungi, demonstrating a broad-spectrum inhibitory activity and showed an attractive potential of protection food products from contamination.

Complete genome sequencing and comparative genomic analysis of strains could provide functional information. To gain insights into genetic elements involved in its antimicrobial activity, we sequenced the entire genome of *L. plantarum* LZ206 in this work. Genomic DNA was isolated using Qiagen DNA extraction kit, and DNA quantity and quality were determined by Agilent 2100 Bioanalyzer. The whole genome of LZ206 was sequenced using a combined strategy of PacBio RSII sequencing and Illumina paired-end sequencing technology. A total of 53,076 reads were yielded with an average length of 7,293 nt. Reads were *de novo* assembled by HGAP 2.3.0, resulting in 4 major contigs with 83.43-fold average coverage (Chin *et*

al., 2013). Automated gene prediction and annotation of the assembled contigs were carried out by the Glimmer Version 3.02 and NCBI Prokaryotic Genome Annotation Pipeline, respectively. Ribosomal RNA genes were identified with RNAmmer1.2 and tRNA genes were searched for using tRNAscan-SE 1.23. As shown in Fig. 2 and Table 2, the complete genome sequence of *L. plantarum* LZ206 was composed of a circular 3.21Mb chromosome, two circular plasmids named as LZ206p2 (33,001 bp) and LZ206p3 (11,652 bp), and a predicted linear plasmid LZ206p1 (6,111 bp). GC content of the complete genome was 44.64% and that of plasmid LZ206p1-p3 were 37.47%, 39.93 % and 41.08 %, respectively. There are 2837 protein-coding genes among the 3092 totally identified genes. 2794 protein-coding genes located on the chromosome, and 5, 28 and 10 were on the plasmids LZ206p1 - LZ206p3, respectively. Each gene was functionally assigned to a category using the following databases: Clusters of Orthologous Groups (COG), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Swiss-Prot and Non-Redundant Protein Database (NR) (Table S1) (Tatusov *et al.*, 2000). 16 rRNA operons, 67 tRNAs, 4 other RNAs and 168 pseudo genes were found in the genome (Table 2).

We hypothesized that antimicrobial activity of *L. plantarum* LZ206 against pathogens was associated with plantaricin biosynthesis gene clusters and their products, class II bacteriocins, as we have found in *L. plantarum* ZJ316 (Li *et al.*, 2016). Indeed, a 19.2 kb putative plantaricin biosynthesis gene cluster was identified, including 6 operons: bacteriocin operons *plnJKL* and *pLNC8 $\alpha\beta$* , regulatory operon *plnA*, transport operon *plnGHSTUVW*, operon *pLNC8IFK* and operon *plnMP* encoding immunity proteins PlnM and PlnP (Fig. 3). Class IIb plantaricin PlnJK and plantaricin NC8 $\alpha\beta$, and also class IIc plantaricin A presursor peptide PlnA could be potentially encoded. PlnA induces transcription of genes organized in operons *plnABCD*, *plnEFI*, *plnJKLR*, *plnMNOP*, and *plnGHSTUV*, therefore the presence of *plnA* in LZ206 and other lactobacilli strains could be associated with the high-level production of plantaricins (Anderssen *et al.*, 1998). Srivastava *et al.* reported PlnJK had antimicrobial activity against pathogenic yeast *Candida albicans* and *Escherichia coli* (Pal and Srivastava, 2014; Sharma and Srivastava, 2014), and we have found that plantaricin NC8 $\alpha\beta$ was very active for controlling and inhibiting the food-borne pathogenic Gram-negative bacteria *Salmonella* spp. (Jiang *et al.*, 2016). There are few *L. plantarum* strains containing genes encoding all three of these bacteriocins: plantaricin NC8 $\alpha\beta$, PlnJK and PlnA. We compared the plantaricin biosynthesis gene

cluster of LZ206 with the four closest evolutionary relatives: *L. plantarum* WCFS1, *L. plantarum* ZJ316, *L. plantarum* subsp. *plantarum* NC8 and *L. plantarum* subsp. *plantarum* P-8. As shown in Fig. 3, WCFS1 contains genes encoding PlnJK and PlnA, but not plantaricin NC8 $\alpha\beta$; ZJ316 and NC8 contain genes encoding plantaricin NC8 $\alpha\beta$ and PlnJK, but not PlnA; while P8 contains genes encoding PlnEF only. Together, *L. plantarum* LZ206 could be a great bactericin producer, potentially producing at least three class II bacteriocins, PlnJK, PlnNC8 $\alpha\beta$ and PlnA, which might explain its antimicrobial activity against pathogenic Gram positive bacteria, Gram negative bacteria and fungi.

The whole genome sequencing of *L. plantarum* LZ206 allows us to better understand molecular basis of its antimicrobial activity and probiotic potentials, and could facilitate its applications to protect the dairy products from pathogens “naturally” by *in situ* fortification. However, more *in vivo* and *in vitro* studies need to be done to establish its safety status and potential health benefits.

Nucleotide sequence accession number. Complete genome sequence of *L. plantarum* LZ206 has been deposited in GenBank under accession number CP015966- CP015969 and the strain has been deposited at the China Center for Type Culture Collection (CCTCC) under accession number CCTCC AB 2016120.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. Phylogenetic tree of *L. plantarum* LZ206. The phylogenetic tree was constructed using software MEGA 6.0 by the neighbour-joining method.

Figure 2. Circular genome map of *L. plantarum* LZ206. The scales indicated the location in Mbp, starting with the initial coding region. From the innermost circles, circle (1) showed the GC skew (G-C/G+C). The value was plotted as the deviation from the average GC skew of the entire sequence. Circle (2) GC content, plotted using a sliding window. Circle (3) denoted tRNA/rRNA. Circle (4, 5) illustrated the CDSs, colored according to COG function categories, 4 was backward strand, 5 was forward strand. Circle (6, 7) indicated m4C and m6A sites in CDS/rRNA/tRNA, 6 was backward strand, 7 was forward strand. Circle (8) demonstrated m4C and m6A sites in the intergenomic regions.

Figure 3. Genetic map of plantaricin biosynthesis gene cluster of *L. plantarum* LZ206 and other *L. plantarum* strains. The pln genes in strains *L. plantarum* LZ206 (CP015966), *L. plantarum* WCFS1 (NC_004567), *L. plantarum* subsp. *plantarum* P-8 (NC_021224), *L. plantarum* ZJ316 (CP004082), *L. plantarum* 16 (NC_021514) and *L. plantarum* subsp. *plantarum* NC8 (AGRI00000000) were showed by arrows with different colors corresponding to each operon.

Tables

Table 1 Inhibition spectrum of culture supernatants of *L.plantarum* LZ206.

Indicator strains	G ⁺ /G ⁻	Inhibitory effects [*]
<i>Micrococcus luteus</i>	G ⁺	++
<i>Bacillus subtilis</i>	G ⁺	++
<i>Listeria monocytogenes</i>	G ⁺	+++
<i>Staphylococcus aureus</i>	G ⁺	++
<i>Staphylococcus carnosus</i> pCA44	G ⁺	++
<i>Staphylococcus simulans</i>	G ⁺	++
<i>Staphylococcus citreus</i>	G ⁺	+
<i>Lactococcus lactis</i> NZ9000	G ⁺	—
<i>Lactobacillus plantarum</i> ZJ316	G ⁺	—
<i>Lactobacillus plantarum</i> LZ206	G ⁺	—
<i>Escherichia coli</i>	G ⁻	+++
<i>Salmonella enterica</i> subsp. <i>enterica</i>	G ⁻	++++
<i>Pseudomonas aeruginosa</i>	G ⁻	—
<i>Erwinia persicina</i>	G ⁻	+++
<i>Candida albicans</i>	fungus	+++

^{*}Inhibition zone in diameter (mm): +++++, 20-25; +++, 15-20; ++, 10-15; +, 5-10; —, no inhibition activity.

Table 2
Features of *Lactobacillus plantarum* LZ206 genome.

Attributes	Values
Genome size (bp)	3,212,951
GC content (%)	44.64%
Plasmid	3 (2 in circular form)
rRNAs	16
tRNAs	67
Other RNAs	4
Proteins	2837
Predicted genes	3092 (310 in plasmid)
Pseudo genes	169

Figure1

0.01

Figure2

Figure3