Identification of salt tolerance-related genes of *Lactobacillus plantarum* D31 and T9 strains by genomic analysis

Wenting Yao 1†, Lianzhi Yang 1†, Zehuai Shao 1, Lu Xie 2 and Lanming Chen 1*

**Abstract**

**Purpose:** The aim of this study was to identify salt tolerance-related genes of *Lactobacillus plantarum* D31 and T9 strains, isolated from Chinese traditional fermented food, by genomic analysis.

**Methods:** Tolerance of *L. plantarum* D31 and T9 strains was evaluated at different stress conditions (temperatures, acid, osmolality, and artificial gastrointestinal fluids). Draft genomes of the two strains were determined using the Illumina sequencing technique. Comparative genomic analysis and gene transcriptional analysis were performed to identify and validate the salt tolerance-related genes.

**Results:** Both *L. plantarum* D31 and T9 strains were able to withstand high osmotic pressure caused by 5.0% NaCl, and *L. plantarum* D31 even to tolerate 8.0% NaCl. *L. plantarum* D31 genome contained 3,315,786 bp (44.5% GC content) with 3,106 predicted protein-encoding genes, while *L. plantarum* T9 contained 3,388,070 bp (44.1% GC content) with 3,223 genes. Comparative genomic analysis revealed a number of genes involved in the maintenance of intracellular ion balance, absorption or synthesis of compatible solutes, stress response, and modulation of membrane composition in *L. plantarum* D31 and or T9 genomes. Gene transcriptional analysis validated that most of these genes were coupled with the stress-resistance phenotypes of the two strains.

**Conclusions:** *L. plantarum* D31 and T9 strains tolerated 5.0% NaCl, and D31 even tolerated 8.0% NaCl. The draft genomes of these two strains were determined, and comparative genomic analysis revealed multiple molecular coping strategies for the salt stress tolerance in *L. plantarum* D31 and T9 strains.

**Keywords:** *Lactobacillus plantarum*, Salt stress tolerance, Genome sequence, Comparative genomics, Gene transcription, Traditional fermented food

**Introduction**

Lactic acid bacteria (LAB) are generally recognized as safe food-grade microorganisms. Numerous previous studies have revealed their beneficial effects on human health, such as maintaining the balance of gastrointestinal microbial community, acting against pathogenic microorganisms, and enhancing innate and adaptive immune responses (Chen et al. 2014; Liu et al. 2018; Nazir et al. 2018). *Lactobacillus plantarum* is one of the most widely applied LAB in the food industry. The bacterium is found to thrive in indigenous microbiota commonly found in fermented food (Zago et al. 2011) and can competitively inhibit pathogenic bacteria growth during fermentation (Molin 2001). LAB chosen for commercial purposes must challenge adverse conditions encountered in industrial processes, such as heat, cold, acidity, and high concentrations of NaCl (Bucka-Kolendo and Sokolowska 2017). Many fermented food are made with the salt, by which osmotic stress is often a significant challenge for microorganisms surviving in fermentation processes (El-Gendy et al. 1983; Yamani et al. 1998; Prasad et al. 2003; Rao et al. 2004). Possible mechanisms of the regulation of intracellular osmotic pressure in LAB have been mentioned,
such as (1) exclusion of Na\(^+\) ion from cells, (2) accumulation of compatible solutes, and (3) changes of cell membrane composition. In the past decade, a number of LAB strains have been subjected for genome sequencing to further address their physiological functions and environmental adaptation mechanisms, along with the development of genome sequencing technologies. To date, more than 50 complete genome sequences of \textit{L. plantarum} strains are available in the GenBank database (http://www.ncbi.nlm.nih.gov/genome/). Genomic diversity and versatility of \textit{L. plantarum} have been reported (Siezen and van Hylckama Vlieg 2011; Jiang et al. 2018; Evanovich et al. 2019). Nevertheless, among these strains, only \textit{L. plantarum} ST-III has been reported to be able to survive in De Man Rogosa and Sharp (MRS) medium with 7.5% NaCl (Chen et al. 2012). A \textit{kdp} gene cluster encoding a high-affinity K\(^+\)-transport system was identified from a 53.56-kb plasmid pST-III in \textit{L. plantarum} ST-III, which was found to contribute to its viability under hyperosmotic conditions (Chen et al. 2012). Recently, Wang et al. reported that \textit{L. plantarum} ATCC 14917 was also able to survive in the MRS medium with 6.0% NaCl. The expression of eleven genes were upregulated in this bacterium to respond to the salt stress, including those involved in carbohydrate metabolism, transcription and translation, fatty acid biosynthesis, and primary metabolism (Wang et al. 2016).

\textit{L. plantarum} with novel functional properties is of interest to both academic institution and food industry. In our prior studies, a number of LAB strains were isolated from Chinese traditional fermented food and identified and characterized by Xu et al. (Xu et al. 2016). Among these, \textit{L. plantarum} D31 and \textit{L. plantarum} T9 strains showed high levels of antioxidant and bile salt hydrolase activities in in vitro tests (Xu et al. 2016). In this study, tolerance of these two strains to various stress conditions was further evaluated, and the resulting data showed that both \textit{L. plantarum} D31 and T9 strains were able to survive in the MRS medium with 5.0% NaCl, and \textit{L. plantarum} D31 even tolerated to 8.0% NaCl. Thus, draft genome sequences of these two strains were determined using the Illumina sequencing technique in order to get genomic insights into possible molecular mechanisms of the salt tolerance of \textit{L. plantarum}.

**Materials and methods**

\textit{L. plantarum} strains and cultural conditions

\textit{L. plantarum} D31 and T9 strains, isolated from Chinese traditional fermented food Dongbei kimichi and milk tofu, respectively, have been identified and characterized in our previous research (Xu et al. 2016). In this study, these two strains were individually inoculated from our laboratory stock at –80 °C into the MRS medium (pH 6.8, Beijing Land Bridge Technology, Beijing, China) and incubated at 37 °C for 24 h under anaerobic conditions as described previously (Xu et al. 2016). Bacterial cells were harvested by centrifugation at 4000 r/min for 10 min and washed three times using the sterile 1 \times phosphate-buffered saline (PBS, pH 7.0, Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., Shanghai, China). Then, cell pellets were resuspended with an appropriate amount of sterile deionized water and used as the inoculum (10\(^7\)–10\(^8\) colony forming unit (CFU)/ml) in the further analyses. Cell density was determined using a multimode microplate reader (Spectrafluor Plus, BioTek Instruments, Winooski, VT, USA), and the OD\(_{600nm}\) value was used as a related parameter for the amount of bacteria biomass (Dahroud et al. 2016).

**Stress conditions**

\textit{L. plantarum} D31 and T9 strains were individually inoculated in the MRS medium (pH 6.8) at different temperatures (15 °C, 20 °C, 25 °C, 37 °C, and 45 °C) for 72 h, and their growth curves were measured according to the method described previously (Li et al. 2017). Acid tolerance of \textit{L. plantarum} D31 and T9 strains was evaluated according to the method described previously (Lee et al. 2014) with slight modification. The cell suspension of \textit{L. plantarum} D31 and T9 strains was individually inoculated into acidic MRS broth (pH 2.0 to 7.0) and incubated at 37 °C for 24 h. Tolerance of \textit{L. plantarum} D31 and T9 strains to different concentrations of NaCl (0.0%, 5.0%, 8.0%, 10.0%, 12.0%, and 15.0%) was determined according to the method described previously (Xin et al. 2014). Bile salt tolerance of \textit{L. plantarum} D31 and T9 strains was evaluated according to the method described previously (Shehata et al. 2016) with slight modification. The cell suspension of \textit{L. plantarum} D31 and T9 strains was individually inoculated into the MRS broth containing different concentrations of bile salt (0.0%, 0.05%, 0.1%, 0.2%, and 0.3%) (Beijing Land Bridge Technology, Beijing, China) and incubated at 37 °C for 24 h. Growth curves of these two strains at different acid, osmotic pressure, and bile salt conditions were individually determined as described above. Tolerance of \textit{L. plantarum} D31 and T9 strains to artificial gastric and intestinal fluids was determined according to the method described previously (Zhang et al. 2016) with slight modification. The cell suspension of \textit{L. plantarum} D31 and T9 strains was individually inoculated (10%, v/v) into the artificial gastric and intestinal fluids and incubated at 37 °C for 180 min and 240 min, respectively. The viable cell count assay was performed as described previously (Zhang et al. 2016). Artificial gastric fluid contained 0.35% pepsin and 0.2% NaCl in 100 ml of distilled water. The solution pH was adjusted to 2.0 with 1 mol/l HCl (Zhang et al. 2016). Artificial intestinal fluid contained 0.1% trypsin, 1.1% NaHCO\(_3\), and 0.2% NaCl in 100 ml of
distilled water. The solution pH was adjusted to 6.8 with 0.6 mol/l NaOH (Zhang et al. 2016). The artificial gastrointestinal fluids were sterilized by filtering through a 0.22-μm membrane (Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., Shanghai, China).

Genomic DNA preparation
Genomic DNA was prepared using a MiniBEST DNA extraction kit (Japan TaKaRa BIO, Dalian Company, China) following the manufacturer’s instructions. Extracted DNA samples were analyzed by electrophoresis with a 0.7% agarose gel and visualized and recorded using a UVPEC3 Imaging system (UVP LLC, UpLand, CA, USA) (Figure S1). The DNA concentration and purity (A260/A280) were measured using a multi-mode microplate reader (BioTek Instruments, Inc., Winooski, VT). Only pure genomic DNA samples (a 260/280 nm absorbance ratio of 1.8–2.0) were used for genome sequencing.

Genome sequencing and assembly
The genome sequencing of \emph{L. plantarum} D31 and \emph{L. plantarum} T9 strains was carried out at Meiji Biological Medicine technology Ltd. (Shanghai, China) and Beijing Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) using a Genome Sequencer Illumina HiSeq Xten platform (Illumina, CA, USA), respectively. Sequence quality was analyzed using the FastQC software (Brown et al. 2017). Raw sequencing reads were trimmed and assembled using the SOAPdenovo v2.04 software (http://soap.genomics.org.cn/).

Genome annotation
Protein-encoding genes, tRNA genes, and rRNA genes were predicted using the GeneMarks (version 4.17) (Besemer et al. 2001) and Glimmer (version 3.02) (Delcher et al. 2007), tRNA scan-SE (version 1.3.1) (Lowe and Eddy 1997), and RNAmmer (version 1.2) (Lagesen et al. 2007) software, respectively. Protein functions were predicted against the Clusters of Orthologous Groups (COG) database (Tatusov et al. 2001). Prophage-associated genes were predicted using a Prophage finder software (http://phast.wishartlab.com/). Clustered regularly interspaced short palindromic repeats (CRISPRs) were detected using the CRISPRFinder software (Grissa et al. 2007). Pfam domain, signal peptide and transmembrane domain, and transmembrane helices were predicted using the Web CD-Search Tool (Marchler-Bauer et al. 2015), SignalP 4.1 Serve (Petersen et al. 2011), and TMHMM (Krogh et al. 2001) software, respectively. Potential virulence factors were detected against the Virulence Factor Database (http://www.mgc.ac.cn/VFs/). Antibiotic resistance genes were searched in the Antibiotic Resistance Genes Database (Gupta et al. 2014).

Comparative genome analysis
Comparative genome analysis was performed between \emph{L. plantarum} D31 and T9 strains, and 50 \emph{L. plantarum} strains whose complete genome sequences (Table S1) were available by 31 March 2018. The complete genome sequences were retrieved from the NCBI genome database (http://www.ncbi.nlm.nih.gov/genome). The Blastcluster software (http://www.ncbi.nlm.nih.gov/) was used for pan-genome analysis. Orthologous genes were analyzed using the CD-HIT software (Fu et al. 2012). Orthologous proteins were assigned only for proteins sharing both 60% amino acid identity and 80% sequence coverage, and strain-specific genes present in one genome had no significant BLAST hit against reference groups at E ≤ 1e-5. Homologous sequences of each gene were aligned using the MUSCLE software (Edgar 2004). A phylogenetic tree was constructed and viewed using the PHYML (Guindon and Gascuel 2003) and EvoView (Zhang et al. 2012) software, respectively. Bootstrap values above 50% were obtained from 1000 bootstrap replications.

Quantitative real-time reverse transcription PCR (qRT-PCR)
Selected salt resistance-associated genes were validated by qRT-PCR assay as described previously (Sun et al. 2014; Zhu et al. 2017). \emph{L. plantarum} D31 and T9 strains were incubated in the MRS medium supplemented with 8% and 5% NaCl, respectively, and cell culture grown to logarithmic growth phase was harvested by centrifugation as described above. Total RNA was prepared using the RNasy-Free DNase Set (QIAGEN Biotech Co., Ltd., Hilden, Germany) according to the manufacturer’s instructions. The DNA was removed from the samples using RNase-Free DNase Set (QIAGEN, Hilden, Germany). RNA samples were analyzed by 1% agarose gel electrophoresis at 100 V for 30 min (Figure S2), and its quality and quantity was assessed using a multi-mode microplate reader (BioTek Instruments, Inc., Winooski, VT). The reverse transcription reaction was performed using the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Japan TaKaRa BIO, Dalian Company, Dalian, China) according to the manufacturer’s protocol. RT-PCR was performed using the TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) (Japan TaKaRa BIO, Dalian Company, Dalian, China) according to the manufacturer’s instructions. A 20-μl reaction volume contained 10 μl TB Premix Ex TaqTM II, 0.4 μl of each of the oligonucleotide primers (10 μmol), 0.4 μl of ROX Reference DyelII, 2 μl of cDNA template, and appropriate volume of sterile DNase/RNase-Free deionized water. All RT-PCR reactions were performed in a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles...
of denaturation at 95 °C for 15 s, and primer annealing at 60 °C for 60 s. The 16S rRNA gene was used as the reference gene, as previously described (Zhu et al. 2017). The expression of the 16S rRNA gene in L. plantarum D31 and T9 strains grown to the logarithmic growth phase in MRS medium supplemented with no NaCl was used as a reference/baseline, respectively. The data were analyzed using the Applied Biosystems 7500 software, and the relative expression ratio was calculated for each target gene by using the delta-delta threshold cycle (Ct) method (Livak and Schmittgen 2001). Oligonucleotide primers were designed using the Primer 5.0 software (http://www.premierbiosoft.com/) and synthesized by Shanghai Sangon Biological Engineering Technology Services Co. Ltd. (Shanghai, China) (Table 1). All determinants were performed in triplicate.

**Genome sequence accession numbers**
The draft genomes of L. plantarum D31 and L. plantarum T9 strains were deposited in GenBank under the accession numbers RCPFP00000000 and RBAI00000000, respectively.

**Results and discussion**
To date, more than 50 complete genome sequences of L. plantarum strains are available in public databases; nevertheless, little genome information is for the salt-tolerant LAB. Moreover, only few L. plantarum strains have been reported to tolerate osmotic stress, e.g., L. plantarum ST-III and L. plantarum ATCC 14917 strains (El-Gendy et al. 1983; Rao et al. 2004; Chen et al. 2012; Vasyliuk et al. 2014; Xin et al. 2014). In this study, for the first time, we evaluated tolerance of L. plantarum D31 and L. plantarum T9 strains, isolated from Chinese traditional fermented food, to different stress conditions (temperatures, acid, osmolality, and artificial gastrointestinal fluids).

**Survival of L. plantarum D31 and T9 strains at different temperatures**
Growth curves of L. plantarum D31 and T9 strains incubated at different temperatures (15 to 45 °C) were determined, and the resulting data are illustrated in Fig. 1a–c. No distinct difference in growth was observed between these two strains at an optional growth temperature of 37 °C (Fig. 1c). Moreover, at the lower (15 °C) or higher (45 °C) temperatures, the growth of these two strains was obviously inhibited, and their OD_{600nm} values showed no significant change for 72 h (figure not shown). Nevertheless, when incubated at 20 °C, L. plantarum T9 still grew well but with a long lag phase (18 h) and reached stationary growth phase (SGP) at 54 h, whereas the growth of L. plantarum D31 was retarded with a longer lag phase (54 h) and entered into the SGP at 72 h (Fig. 1a). Similar growth phenotypes of these two strains were observed at 25 °C as did at 20 °C (Fig. 1b), suggesting the medium-temperature growth feature of L. plantarum T9 and D31 strains.

**Survival of L. plantarum D31 and T9 strains at acidic pH conditions**
Tolerance of L. plantarum under acid stress has been reported (Huang et al. 2016). In this study, as shown in Fig. 2, L. plantarum T9 and D31 strains were able to grow at pH 5.0 and optimally at pH 6.0 to 7.0, consistent with previous research (Nyanga-Koumou et al. 2012). Additionally, L. plantarum D31 did not grow at pH 4.0, whereas L. plantarum T9 was observed to grow slowly at this pH condition. Moreover, no cell growth of these two strains was observed under more acidic conditions with pH values lower than 3.0 (Fig. 2).

**Tolerance of L. plantarum D31 and T9 strains to different concentrations of NaCl**
Tolerance of L. plantarum D31 and T9 strains to different concentrations of NaCl was determined, and the resulting data are illustrated in Fig. 3. The two strains were found to grow well in the MRS medium supplemented with 0.0–5.0% NaCl at 37 °C. Also, L. plantarum D31 was able to grow at 8.0% NaCl, whereas the growth of L. plantarum T9 was obviously inhibited at this NaCl concentration. No cell growth of these two strains was found when the NaCl concentration was more than 8.0% (Fig. 3).

**Survival of L. plantarum D31 and T9 strains in different concentrations of bile salt**
Previous research has reported bile-tolerant L. plantarum and key proteins by comparative proteomic analysis (Hamon et al. 2011). In this study, as shown in Fig. 4, bile salt tolerance of L. plantarum D31 and T9 strains was examined. The growth of these two strains was obviously inhibited at 0.05% bile salt. Moreover, neither L. plantarum D31 nor T9 strains could withstand more than 0.1% bile salt (Fig. 4).

**Survival of L. plantarum D31 and T9 strains in artificial human gastric and intestinal fluids**
Tolerance of L. plantarum D31 and T9 strains to artificial gastric and intestinal fluids was also determined. After treated in the artificial gastric fluid for 180 min, the growth of L. plantarum D31 was completely inhibited, while an extremely low survival rate (0.03%) was observed for L. plantarum T9. Likewise, the growth of L. plantarum D31 and T9 strains was also significantly reduced in artificial intestinal fluid for 240 min, and the survival rates were 11.7% and 0.87% for L. plantarum D31 and T9 strains, respectively (figures not shown).
### Table 1

The oligonucleotide primers used in the qRT-PCR assay in this study

| Locus/gene   | Description of encoded protein                  | Sequence (5′–>3′)                      | Predicted product length (bp) | Source     |
|--------------|------------------------------------------------|----------------------------------------|-------------------------------|------------|
| **L. plantarum D31** |                                                |                                        |                               |            |
| *D7Y65_00120* | Potassium transporter Kup                      | F: ATCGTGATGCTTTTAAATTCC              | 166                           | This study |
|              |                                                 | R: AACAAGCAGAAGGCGGAT                |                               |            |
| *D7Y65_03505* | Cell membrane protein                          | F: TGAGCTGGTTTTGCTGAAT              | 124                           | This study |
|              |                                                 | R: CTCACAACCCGCGCTCTTA              |                               |            |
| *D7Y65_06915* | Co-chaperone GroES                             | F: ATGACCGGTGGTGGTTAGAT              | 117                           | This study |
|              |                                                 | R: CTTGATAACTTGACTCGGT              |                               |            |
| *D7Y65_06920* | Chaperonin GroEL                               | F: GCTCTGTGGTTATCGGCTCTCT            | 106                           | This study |
|              |                                                 | R: TACATCAATACCCGCCCCATACC          |                               |            |
| *D7Y65_09830* | Sigma-54 modulation protein                    | F: CAAGCAATTCGGGACTACGT              | 113                           | This study |
|              |                                                 | R: TTAGACCGTGTTGCTGTTG              |                               |            |
| *D7Y65_09835* | Molecular chaperone DnaJ                      | F: ACAGTGGAAAGTGGCCGTAA              | 139                           | This study |
|              |                                                 | R: AAAGTGGACTGGAAAGAAT              |                               |            |
| *D7Y65_13150* | Potassium transporter Kup                      | F: GGCAGAGAAGGCGGAGTAT              | 145                           | This study |
|              |                                                 | R: TTAGACAATGCGGCCAACG            |                               |            |
| *D7Y65_13295* | LysR family                                   | F: TGAAGCGCGTTATTATGGTG              | 149                           | This study |
|              |                                                 | R: GATTGCCGAAGAATTTGACC            |                               |            |
| *D7Y65_02550* | Metal-independent α-mannosidase                | F: AGATACGGGGAATGATTGTTG            | 182                           | This study |
|              |                                                 | R: TACCCAGTGACGGGGAGGCA            |                               |            |
| *D7Y65_07055* | Glycosyl hydrolase family B                   | F: CGGTAGAGTGGTTGGGTTGAA            | 137                           | This study |
|              |                                                 | R: CCAGCAAGTCTGCTTGCT             |                               |            |
| *D7Y65_13825* | Carboxypeptidase                               | F: ATAATAGTCGGCATGTGCTGCT            | 181                           | This study |
|              |                                                 | R: TACTACCCGGTGAGGCGGAC            |                               |            |
| *D7Y65_15155* | Potassium transporter Kup                      | F: AGCAATGGGCCACCCCTAACAC            | 169                           | This study |
|              |                                                 | R: AAGCAATGGGCCAGCAAAACC          |                               |            |
| *D7Y65_15160* | Kdp E                                         | F: CTGACCTTGGTACCGCTCCTC            | 104                           | This study |
|              |                                                 | R: CCGATATGAGGATGGGAGGAC          |                               |            |
| *D7Y65_15165* | KdpD                                         | F: TTTGTTACCGCTGCTTCTT            | 106                           | This study |
|              |                                                 | R: TAACATTAGCGCTGGCCCATCC         |                               |            |
| *D7Y65_15180* | Kdp A                                         | F: AACCACCGTTGTTGGGAGGA           | 142                           | This study |
|              |                                                 | R: GGTATCACCAGTGAGGAGG            |                               |            |
| **L. plantarum T9** |                                                |                                        |                               |            |
| *D7Y66_02210* | Cell membrane protein                          | F: TACCCGGCTCTCTACATCTCT            | 173                           | This study |
|              |                                                 | R: CTCACAACCCGACCTCTTA            |                               |            |
| *D7Y66_03635* | Co-chaperone GroES                             | F: CAATGACCCGCTGGCTGTTTAG          | 108                           | This study |
|              |                                                 | R: CTTGATACCTGTGACTCGGT            |                               |            |
| *D7Y66_03640* | Chaperonin GroEL                               | F: GCTTCTTGGTTACGCCCCTCT           | 113                           | This study |
|              |                                                 | R: TACATCAATACCGGCCCATACC         |                               |            |
| *D7Y66_03675* | Sigma-54 modulation protein                    | F: CAAGCAATTCGGGACTACGT            | 115                           | This study |
|              |                                                 | R: TAGCCGTCTTGGTCGGGTGA            |                               |            |
| *D7Y66_07525* | Potassium transporter Kup                      | F: CTCAGCTATTTCCGCTACGAGG          | 103                           | This study |
|              |                                                 | R: CCATCGTCAGGTTAGCCAGG           |                               |            |
Table 1 The oligonucleotide primers used in the qRT-PCR assay in this study (Continued)

| Locus/gene | Description of encoded protein | Sequence (5′–3′) | Predicted product length (bp) | Source |
|------------|--------------------------------|------------------|-------------------------------|--------|
| D7Y66_08075 | Molecular chaperone DnaJ | F: CCAGCAGGAACCGTCACTTT  
R: CCACCTGGTCCAGATAGGAG | 140 | This study |
| D7Y66_08080 | Molecular chaperone DnaK | F: ACCAAGTGAAGTGAGGCATAA  
R: AAAGTGGAAGCAGAAGAT | 139 | This study |
| D7Y66_11330 | LysR family | F: GATTCTGGAGGACCGCTATAA  
R: GCAACTGCTGAAAGCCTA | 179 | This study |
| D7Y66_13435 | Potassium transporter Kup | F: GCCAGTACAAACCTTGAA  
R: TGGTACGCTGACTGCAACCT | 168 | This study |
| lp_3505 | Acetyl esterase | F: TTGGTATGTAATCGTGGGT  
R: TGTTAGCAGATGGCACCCT | 117 | This study |
| 16S RNA | | F: AAGGGTTTCCGCTGCTAAAA  
R: TGCACTGAAAGTGCTCCAGTT | 247 | Sun et al. 2014 |

* forward primer, R reverse primer; *The genes detected in L. plantarum D31 strain

Genome features of L. plantarum D31 and T9 strains

Draft genomes of L. plantarum D31 and T9 strains were determined using the Illumina sequencing technique in order to get insights into possible molecular mechanisms of their salt stress tolerance. This analysis generated 8,987,722 and 6,246,667 reads for L. plantarum D31 and T9 with sequencing depth of 406-fold and 277-fold, respectively. The L. plantarum D31 draft genome contains 3,315,786 bp with a GC content of 44.5%. The final assembly comprised 72 scaffolds. Total 3251 genes were predicted, including 3106 predicted protein-coding genes and 48 RNA genes (Table 2). Among the predicted genes, about 73.7% had a predicted function, and 55.1% were assigned to COG (Table S2). Two intact prophage elements (76.5 kb, scaffold 1: 73,403–149,937 bp; 40.8 kb, scaffold 6: 16,933–57,799 bp) and one CRISPR repeat array (37 bp, scaffold 52: 11,856–12,956 bp) were identified in L. plantarum T9 genome, respectively.

Additionally, no virulence gene was identified in L. plantarum D31 and T9 draft genomes. A potential antibiotic gene baca encoding a bacitracin resistance protein (D7Y65_11270, D7Y66_07705) was identified in the two genomes.

The features of these two draft genomes are summarized in Table 2. The draft genomes of L. plantarum D31 and T9 were submitted to GenBank under the accession numbers RCFP00000000 and RBAI00000000, respectively.

Phylogenetic relatedness of L. plantarum strains

As shown in Fig. 5, a phylogenetic tree was constructed, based on 151,630 homologous amino acid sequences...
identified from the 52 *L. plantarum* genomes analyzed in this study, among which complete genome sequences of 50 *L. plantarum* strains were available and retrieved from the GenBank database. This analysis revealed three distinct clusters, designated cluster α, β, and γ. *L. plantarum* D31 and T9 genomes were classified as two singletons (cluster α and cluster β). They were phylogenetically distant from the other *L. plantarum* genomes that were grouped into cluster γ. The cluster γ was further classified into two subclusters I and II, including 13 and 36 genomes, respectively, which were recovered from diverse sources, such as the human saliva and gut, fermented fish, pickle, stinky tofu, and cow milk. Additionally, *L. plantarum* D31 and T9 genomes were distant from the salt-tolerant *L. plantarum* ST-III (ASM14881V1).

**Strain-specific genes in *L. plantarum* D31 and T9 genomes**

Based on the 52 *L. plantarum* genome sequences analyzed in this study, comparative genomic analysis revealed 173 strain-specific genes in *L. plantarum* D31 genome, of which 167 genes encoded hypothetical proteins, suggesting possible strain-specific mechanisms of stress tolerance and/or niche adaptation. The remaining strain-specific genes were involved in cell wall biosynthesis, carbohydrate metabolism, and stress response, e.g., the L-fructose isomerase (D7Y65_15415), pilus assembly protein (D7Y65_15865), bleomycin binding protein Ble-MBL (D7Y65_15890), molecular chaperone DnaJ (D7Y65_15980), single-stranded DNA-binding protein (D7Y65_16040), and conjugal transfer protein TraG (D7Y65_16080). Likewise, *L. plantarum* T9 had 112 strain-specific genes; however, most of which (111 genes) encoded hypothetical proteins, and one encoded a helix-turn-helix domain-containing protein (D7Y66_00865).

**Genomic insights into possible mechanisms of the salt tolerance of *L. plantarum* D31 and T9 strains**

*Recovery of intracellular ion balance*

The sodium/proton (Na⁺/H⁺) reverse transporter on cytoplasm membrane is the main way of microbial efflux of Na⁺, which regulates intracellular pH homeostasis (Padan et al. 2005). It has been reported that *L. plantarum* 5-2 genome contained eight genes encoding...
the Na+/H+ antiporters (Liu et al. 2015). In this study, comparative genomic analysis revealed at least ten, nine, and six genes encoding Na+/H+ antiporters in *L. plantarum* D31, T9, and ST-III genomes, respectively. Potassium (K⁺) is the most abundant ion in bacterial cytoplasm and plays a pivotal role in ion homeostasis (Epstein 2003). Previous research has indicated that the *kdp* system in *L. plantarum* ST-III enabled the bacterium growing in the presence of curing salts (7.5% NaCl) (Chen et al. 2012). In the *kdp* system, the sensor kinase KdpD and the response regulator KdpE controlled the induction of the *kdpABC* operon in response to an osmotic upshift (Peddie et al. 1994; Grissa et al. 2007; Petersen et al. 2011). In this study, a typical *kdpABCDE* gene locus was also identified in *L. plantarum* D31 genome (D7Y65_15160 to D7Y65_15180), which had high sequence similarity (95%) with *kdp* genes (YP_003927890.1 to YP_003927894.1) in *L. plantarum* ST-III genome. The *kdp* cluster was also identified in another *L. plantarum* GB-LP3 genome, but absent from *L. plantarum* T9 draft genome. It has also been reported that K⁺ is accumulated far above the normal level in the primary response in *Escherichia coli* to the osmotic upshift (Heermann et al. 2009). In *E. coli*, *kup* is the major K⁺ uptake system under hyperosmotic stress and low pH conditions (Zakharyan and Trchounian 2001). In this study, upstream of the *kdp* gene cluster, a K⁺-transport system gene *kup* was identified in *L. plantarum* D31 genome (D7Y65_15155), showing 94% sequence similarity with *kup* genes (WP_013356293.1) in *L. plantarum* ST-III, which may act as the major K⁺ uptake system in the MRS medium with 7.5% NaCl (Chen et al. 2012). Moreover, another two *kup* genes (D7Y65_13150, D7Y65_00120) were also identified from *L. plantarum* D31 genome, while only two were identified from *L. plantarum* T9 (D7Y66_07525, D7Y66_13435).

**Table 2** *L. plantarum* D31 and T9 genome statistics

| Feature                           | *L. plantarum* D31 | Percentage of total | *L. plantarum* T9 | Percentage of total |
|----------------------------------|--------------------|---------------------|-------------------|---------------------|
| Genome size (bp)                 | 3,315,786          | 100.00              | 3,388,070         | 100.00              |
| DNA coding (bp)                  | 2,777,217          | 83.76               | 2,788,032         | 82.29               |
| DNA G + C (bp)                   | 1,474,530          | 44.47               | 1,494,478         | 44.11               |
| DNA scaffold                     | 72                 |                     | 168               |                     |
| Total gene                       | 3251               | 100.00              | 3515              | 100.00              |
| Protein-coding gene              | 3106               | 95.54               | 3223              | 91.69               |
| RNA gene                         | 48                 | 1.48                | 77                | 2.19                |
| Pseudo gene                      | 97                 | 2.98                | 215               | 6.12                |
| Genes with function prediction   | 2450               | 75.36               | 2590              | 73.68               |
| Genes assigned to COG            | 1896               | 58.32               | 1935              | 55.05               |
| Genes with Pfam domain           | 2530               | 77.82               | 2578              | 73.34               |
| Genes with signal peptide        | 138                | 4.24                | 143               | 4.07                |
| Genes with transmembrane helices | 845                | 25.99               | 876               | 24.92               |
| CRISPR repeat                    | 1                  | 1                   | 1                 | 1                   |
| Intact prophage                  | 1                  | 1                   | 2                 | 2                   |
Absorption or synthesis of compatible solutes

Accumulation of certain compatible solutes (e.g., glycine and betaine) is a common metabolic adaptation found in diverse species (Oshone et al. 2017). The osmotic function of a compatible solute depends on the degree of methylation and length of the hydrocarbon chain (Peddie et al. 1994). It has been reported that the electrolyte-mediated osmolality up-shifts led to the accumulation of compatible solutes (Glaasker et al. 1998). In this study, genes involved in absorption or synthesis of compatible solutes were identified in L. plantarum D31 and T9 genomes. For instance, the genes encoding glycine/betaine/carnitine ABC transporters (opuABCD, choSQ) were identified in L. plantarum D31 (D7Y65_03265 to D7Y65_03280) and T9 (D7Y66_01970 to D7Y66_01985) genomes, respectively, which had high sequence similarity (99%) with the corresponding genes in L. plantarum ST-III (Kleerebezem et al. 2003). Moreover, the gene involved in nitrate/sulfonate/bicarbonate ABC transporter was identified in L. plantarum D31 (D7Y65_10050) and T9 genomes (D7Y66_08295), respectively, which was upregulated in response to salt stress in L. plantarum ST-III (Kleerebezem et al. 2003). These genes were involved in multi-component binding-protein-dependent transport systems for glycine, betaine, and carnitine, and accumulated to high levels in the cell in response to increased external osmolality (Huang et al. 2010). Additionally, proline is essential for primary metabolism in salt stress and plays a molecular chaperone role in maintaining the pH of the cytosolic redox status of the cell (Kido et al. 2013). Previous research has indicated that proABC genes are related to the accumulation of proline and enable bacteria to withstand high osmotic pressure (Mahan and Csonka 1983). In this study, a proABC gene cluster was identified in L. plantarum D31 (D7Y65_02230, D7Y65_02235, D7Y65_14760) and T9...
Modulation of cell membrane

The composition of the cell envelope plays an important role in bacterial osmo-adaptation (Sun et al. 2014). Salt stress triggers alterations in structure and composition of the cell peptidoglycan layer (Piuri et al. 2010). In this study, comparative genomic analysis also revealed the genes involved in the modulation of cell membrane in L. plantarum D31 and T9 genomes. For instance, the genes encoding a lysyl-phosphatidylglycerol (D7Y65_14200, D7Y66_03470) and a phosphatidylglycerol (D7Y65_14200/D7Y65_07030, D7Y66_03470/D7Y66_15490) were identified in L. plantarum D31 and T9 genomes, respectively. Moreover, the gene encoding a membrane protein (D7Y65_03505, D7Y66_02210) was also identified in L. plantarum D31 and T9 genomes, which had 50% sequence similarity with the gene (CCI6_RS12035) in Frankia sp. Cc16, which was involved in cell wall/membrane/envelop biosynthesis and upregulated under the salt stress (Oshone et al. 2017). L. plantarum D31 and T9 also contain the genes encoding a 1-acylglycerol-3-phosphate O-acyltransferase (D7Y65_09990, D7Y66_08235) and a phosphatidylglycerophosphatase A (D7Y65_11075, D7Y66_07900), which are involved in cell wall modification to response to the salt stress in Frankia strains (Oshone et al. 2017).

Stress response

Previous studies have indicated that induction of one-component regulatory systems (e.g., GroES-GroEL and DnaK-DnaJ) is related with acid, ethanol, cold, osmotic, starvation, and temperature stresses (Sugimoto et al. 2008). DnaK was first found in heat stress in E. coli (Arsene et al. 2000), but it can also be overexpressed under salt stress (Bucka-Kolendo and Sokolowska 2017). In this study, the genes encoding one-component regulatory systems DnaK-DnaJ and GroES-GroEL were identified in L. plantarum D31 (D7Y65_09835 to D7Y65_09830, D7Y65_06915 to D31_D7Y65_06920) and T9 genomes (D7Y66_08080 to D7Y66_08075, D7Y66_03635 to D7Y66_15720), respectively. A gene encoding GroES-like protein (D7Y65_06915) was also identified in L. plantarum D31 and ATCC14917 genomes. Qin et al. reported that a marine bacterium Zunongwangia profunda (MCCC 1A01486) showing extreme salt tolerance has a cold-active and salt-tolerant α-amylase (AmyZ) belonging to glycoside hydrolase family 13 (Qin et al. 2014). Kang et al. also reported that the gene lj0569 encoding a conserved domain of glycoside hydrolase family 31 was present in Lactobacillus johnsonii NCC533, which was found to survive in a high concentration of NaCl (29 g/l) (Kang et al. 2009). The genes encoding proteins that...
have conserved domains of glycoside hydrolase families 125 and 8 were identified in *L. plantarum* D31 genome (D7Y65_02550, D7Y65_07055), which had 99% sequence similarity with corresponding genes (WP_013356072.1, WP_033099061.1) in salt-tolerant *L. plantarum* ST-III.

**Regulators**

Previous studies have revealed important roles of regulators in stress tolerance of *L. plantarum* (Wang et al. 2016; Jia et al. 2018). *L. plantarum* D31 draft genome contained approximately 229 genes encoding transcriptional or response regulators, which represented approximately 7.4% of its protein-encoding genes, while *L. plantarum* T9 contained approximately 208 such genes, which represented approximately 6.5% of its protein-encoding genes. These genes may modulate global regulatory networks that are essential for bacterial adaptation to changing environment. For instance, several genes encoding transcriptional factors of GntR, TetR, Crp/Fnr, and LysR families were identified in *L. plantarum* D31 and T9 genomes (Table S3), which have been implicated in bacteria stress responses including heat and osmotic shock (Ramos et al. 2005). The gene (D7Y65_13295, D7Y66_11330) encoding transcriptional regulator of the LysR family was identified in *L. plantarum* D31 and T9 genomes, which shared 61.1% similarity at amino acid sequence level with the gene (CCI6_RS20460) in a salt stress-tolerant *Frankia* sp. Cc6 strain. Genes encoding a RNA polymerase sigma factor RpoD (D7Y65_04790, D7Y66_09275), an S-adenosylmethionine synthetase (D7Y65_12145, D7Y66_14505), a DNA-directed RNA polymerase subunit beta (D7Y65_08230, D7Y66_06445), and an amino acid permease (D7Y65_06925, D7Y66_03645) were also identified in *L. plantarum* D31 and T9 genomes, respectively. The expression of these genes was upregulated in *Frankia* sp. Cc6 grown in 1000 mmol/l NaCl (Oshone et al. 2017). In addition, many differentially expressed proteins responding to 6.0% NaCl stress were identified in *L. plantarum* ATCC14917 (Wang et al. 2016), among which the genes encoding a triosephosphate isomerase (D7Y65_07215, D7Y66_03905), a glyceraldehyde-3-phosphate dehydrogenase (D7Y65_07205, D7Y66_03895), a fructose-bisphosphate aldolase (D7Y65_00895, D7Y66_11760), a trigger factor (D7Y65_10490, D7Y66_10765), a carbamoyl phosphate synthase large subunit (D7Y65_06465, D7Y66_02930), an orotate phosphoribosyltransferase (D7Y65_15300).

**Fig. 7** Possible salt tolerance mechanisms of *L. plantarum* D31 and T9 strains. CH$_2$O, carbohydrate metabolism; GBC, glycine/betaine/carnitine ABC transporters; kdp, kup, K$^+$ transport systems; Na$^+$/H$^+$ RT, Na$^+$/H$^+$ reverse transport; NFB, nitrate/sulfonate/bicarbonate ABC transporters; Osrp, oxidative stress-related proteins; P, proline; pili, pilus assembly proteins; PK, protein kinases; RR, response regulators; Ssrp, salt stress-related proteins; TR, transcription regulators.
06450, D7Y66_02945), an elongation factor Tu (D7Y65_10495, D7Y66_10760), a glutamyl-tRNA synthase (D7Y65_14120, D7Y66_03390), a S-ribosylhomocysteine lyase (D7Y65_07140, D7Y66_03830), a malonyl CoA-acyl carrier protein transacylase (D7Y65_03550, D7Y66_02255), and a sigma-54 modulation protein (D7Y65_06955, D7Y66_03675) were also identified in L. plantarum D31 and T9 genomes, respectively. In addition, the gene encoding a carboxypeptidase was identified in L. plantarum D31 (D7Y65_13825) and ST-III, which shared 25% amino acid sequence similarity with the protein (WP_010874021.1) directly or indirectly involved in salt sensing of Synechocystis sp. PCC 6803 (Huang et al. 2010).

Transcriptional profiles of salt resistance-associated genes in L. plantarum D31 and T9 strains

To couple the salt tolerance phenotypes of L. plantarum D31 and T9 strains with their salt resistance-associated genes identified by the comparative genomic analysis, we determined transcriptional profiles of 16 representative genes by the qRT-PCR assay. This analysis revealed many differentially expressed genes involved in the salt stress in the two strains (Figure S3, Fig. 6). For instance, when L. plantarum D31 was grown in 8% NaCl, the expression of the kdp gene cluster and its regulators (D7Y65_15160, D7Y65_15165, D7Y65_15180) was remarkably upregulated (2.4–4.5 fold, p < 0.05), suggesting enhanced K⁺ uptake of L. plantarum D31 cells in the stress condition. Likewise, when L. plantarum T9 strain incubated in 5% NaCl, the genes encoding the Na⁺/H⁺ antiporters (D7Y66_07525, D7Y66_13435) and co-chaperone GroES and GroEL (D7Y66_03635, D7Y66_03640) showed higher transcriptional levels (changes ≥ 1.0-fold), implying increased antiporting of Na⁺/H⁺ in L. plantarum T9 cells. Comparison of the transcriptional profiles revealed differentially expressed genes that were synchronously elicited from both L. plantarum D31 and T9 strains in the salt stress. Nevertheless, opposite transcriptional patterns were also observed in the two strains. For instance, the genes encoding the GroES and GroEL (D7Y65_06915, D7Y65_06920) were notably downregulated in L. plantarum D31 (0.14–0.33 fold, p < 0.05). Additionally, Esteban-Torres et al. (2014) have reported a cold-active and salt-tolerant esterase from L. plantarum. In this study, the gene (lp_3505) encoding the esterase was also examined in L. plantarum D31 and T9 strains by the qRT-PCR assay. The resulting data showed that expression of the esterase gene was slightly reduced in L. plantarum D31 (0.32 fold, p < 0.05) and T9 (0.47 fold, p < 0.05) strains, respectively. These results suggested possible strain-specific regulatory mechanisms of L. plantarum in the salt stress.

Overall, both L. plantarum D31 and T9 strains were able to withstand high osmotic pressure caused by 5.0% NaCl, and L. plantarum D31 even to tolerate 8.0% NaCl. Our genomic data, coupled with the previous studies, revealed a complex molecular regulatory network responding to the salt stress in L. plantarum (Fig. 7). The salt resistance-associated genes identified in L. plantarum D31 and L. plantarum T9 genomes fall into at least four distinct categories. One of these is involved in the recovery of intracellular ion balance, e.g., the Na⁺/H⁺ reverse transport and K⁺ transport systems. The genes encoding nitrate/sulfonate/bicarbonate ABC transporters and proline synthesis fall into the second category, which are likely essential for absorption or synthesis of compatible solutes. The third category may contain the genes involved in the regulation of intracellular metabolism balance, e.g., encoding transcriptional factors of GntR, TetR, Crp/Fnr, and LysR families, and a number of response regulators, particularly for the modulation of cell membrane composition changes. The genes involved in the stress response, e.g., encoding one-component regulatory systems DnaK-DnaJ and GroES-GroEL, may go into the fourth category (Fig. 7). The data in this study allowed us to better understand molecular coping strategies for the salt tolerance of L. plantarum. The bacterium with novel functional properties is of interest to both academic institution and food industry. Both L. plantarum D31 and T9 strains showing high level of salt tolerance are promising components for traditional food fermentations.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13213-020-01551-2.

Additional file 1: Table S1. The genome features of the 50 L. plantarum strains analyzed in this study.

Additional file 2: Table S2. The genes classified into 24 COG functional categories in L. plantarum D31 and T9 genomes.

Additional file 3: Table S3. Regulators related to the salt stress in L. plantarum D31 and T9 genomes.

Additional file 4: Figure S1. Agarose (0.7%) gel electrophoresis analysis of genomic DNA extracted from L. plantarum D31 and T9 strains. Lane 1: 1 kb DNA molecular Maker; Lane 2 and 3: genomic DNA samples extracted from L. plantarum D31 and T9 strains, respectively. Electrophoresis was performed at 120 voltage for about 30 min in 1 x TAE buffer (Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., Shanghai, China). (B): The marker ladder photo provided by the manufacturer.

Additional file 5: Figure S2. Agarose (1%) gel electrophoresis analysis of total RNA extracted from L. plantarum D31 and T9 strains. Lane 1 and 3: L. plantarum D31 and T9 strains grown in MRS medium, respectively. Lane 2 and 4: L. plantarum D31 and T9 strains grown in MRS medium supplemented with 8%, and 5% NaCl, respectively.

Additional file 6: Figure S3. Amplification plots of qRT-PCR products derived from the sixteen representative salt resistance-associated genes of L. plantarum D31 (A) and T9 (B) strains. The qRT-PCR assay was performed in a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) (See Materials and methods). L. plantarum D31 and T9 strains grown in MRS medium supplemented with 8%, and 5% NaCl, respectively.
Authors’ contributions

WY, LY, ZS, LX, and LC participated in the design and or discussion of the study. WY, and LY carried out the major experiments and analyses. WY, and LC wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the grants from the Shanghai Municipal Science and Technology Commission (No. 17050502200) and the National Natural Science Foundation of China (No. 31671946).

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1Key Laboratory of Quality and Safety Risk Assessment for Aquatic Products on Storage and Preservation (Shanghai), China Ministry of Agriculture, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, People's Republic of China. 2Shanghai Center for Bioinformation Technology, Shanghai 201203, People's Republic of China.

Received: 29 July 2019 Accepted: 29 January 2020

Publicated online: 03 March 2020

References

Arsene F, Tomoyasu T, Bukau B (2000) The heat shock response of Escherichia coli. Int J Food Microbiol 55:9-18.

Bester K, Lonsdale AT, Borodovsky M (2001) GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29:2607–2618.

Brown J, Pirring M, McCue LA (2017) FQC Dashboard: integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. Bioinformatics 33:3137–3139. https://doi.org/10.1093/bioinformatics/btx373.

Bucka-Kolendo J, Sokolowska B (2017) Lactic acid bacteria stress response to high throughput. Nucleic Acids Res 32:1792–1800. https://doi.org/10.1093/nar/gkm360.

Chen L, Xu S, Pan Y (2014) Diversity of lactic acid bacteria in Chinese traditional fermented foods. In: V Ravi Shankar R, Kamal A (eds). Beneficial microorganisms in fermented and functional foods. CRC Press, London, pp:33–149. https://doi.org/10.1201/b17912.

Dahrouj BD, Mokarram RR, Khubani MS, Hamishehkar B, Bilvaaei AZ, Yousef M, Kafli HS (2016) Low intensity ultrasound increases the fermentation efficiency of Lactobacillus casei subsp. casei ATCC 39392. Int J Biobiotecnol 86:462–467. https://doi.org/10.14388/abp.2017_1496.

Delcher AL, Bratke KA, Powers EC, Salzberg SL (2007) Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679. https://doi.org/10.1093/bioinformatics/btm009.

Epstein W (2003) The roles and regulation of potassium in bacteria. Prog Nucleic Acids Res Mol Biol 75:293. https://doi.org/10.1016/S0079-6603(03)75098-9.

Esteban-Torres M, Manchez JM, de las Rivas B, Muñoz R (2014) Characterization of a cold-active esterase from Lactobacillus plantarum suitable for food fermentations. J Agric Food Chem 62:526–531.2. https://doi.org/10.1021/ jf501493z.

Evanoich E, de Souza Mendonca Mattos PJ, Guerreiro JF (2019) Comparative genomic analysis of Lactobacillus plantarum: an overview. Int J Genomics 2019:4973214. https://doi.org/10.1155/2019/4973214.

Fu LM, Niu BF, Zhu ZW, Wu ST, Li WZ (2012) CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150–3152. https://doi.org/10.1093/bioinformatics/bts565.

Glazer E, Tien FS, Ter Steeg PF, Konings WN, Poolman B (1998) Physiological response of Lactobacillus plantarum to salt and nonelectrolyte stress. J Bacteriol 180:4718–4723.

Grissa I, Vergnaud G, Pourcel C (2007) CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 35:52–57. https://doi.org/10.1093/nar/gkm360.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696. https://doi.org/10.1080/03635500175305.

Huang R, Pan M, Chen C, Shah NP, Tao X, Wei H (2016) Physiological and transcriptional responses to a cold-active esterase from Lactobacillus plantarum strain 5-2 isolated from fermented soybean. Genomics 106:404–413. https://doi.org/10.1016/j.ygeno.2015.07.007.
Liu Y, Alobokan JI, Rhoads JM (2018) Probiotics in autoimmune and inflammatory disorders. Nutrients 10:1537 https://doi.org/10.3390/nu10101537
Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta CT) method. Methods (San Diego, Calif). 25, 402-408. https://doi.org/10.1016/S1046-8196(01)262
Lowe TM, Eddy SR (1997) RfamScan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955-964 https://doi.org/10.1093/nar/25.5.955
Mahan MJ, Csonka LN (1983) Genetic analysis of the proBA org isolated potential probiotic lactic acid bacteria for cholesterol lowering. Int J Mol Sci 15:22539–22562 https://doi.org/10.3390/ijms1522539
Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram U, Rao BS, Kryzlin B, Galperin MY, Fedorova ND, Koonin EV (2001) The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Res 29:22-28 https://doi.org/10.1093/nar/29.1.22
Vasylik OM, Kovalenko NK, Harmasheva IL (2014) Physiological and biochemical properties of the Lactobacillus plantarum, isolated from traditional fermented products of Ukraine. Mikrobiol Z 762–8
Wang PP, Wu Z, Wu J, Fan D, Zeng XG, Cheng KM (2016) Effects of salt stress on carbohydrate metabolism of Lactobacillus plantarum ATCC 14917. Curr Microbiol 73:1–7 https://doi.org/10.1007/s00284-016-1087-8
Xin XI, Wang QQ, Wang XR, Yue YT, R-Na Wu (2014) Isolation and identification of salt-resistant lactic acid bacteria in farmers’ soybean paste. Food Ferment Ind 40:33–40
Xu S, Liu T, Radji CA, Yang J, Chen L (2016) Isolation, identification, and evaluation of new lactic acid bacteria strains with both cellular antioxidant and bile salt hydrolase activities in vitro. J Food Prot 79:1919–1928 https://doi.org/10.1111/j.1574-6968.1994.tb07018.x
Yang MI, Alnabulsi AA, Haddadis MS, Robinson RK (1998) The isolation of salt-tolerant lactic acid bacteria from oxine and bovine milks for use in the production of nabulsi cheese. Int J Dairy Technol 51:86–99 https://doi.org/10.1111/j.1365-2028.1998.tb06433.x
Zago M, Fornasari ME, Carminati O, Burns P, Suárez V, Vindelora G, Reinheimer J, Giraffa G (2011) Characterization and probiotic potential of Lactobacillus plantarum strains isolated from cheeses. Food Microbiol 28:1033–1040 https://doi.org/10.1111/j.1574-6968.2011.01209.009
Zakharyan E, Tchrourian A (2001) K+ influx by Kup in Eschrichia coli is accompanied by a decrease in H+ efflux. FEMS Microbiol Lett 204:61–66 https://doi.org/10.1111/j.1574-6968.1994.tb06407.x
Zhang B, Wang Y, Tan Z, Li Z, Jiao Z, Huang Q (2016) Screening of probiotic activities of Lactobacillus plantarum isolated from traditional Tibetan Qula, a raw yak milk cheese. Asian Austral J Anim 29:1490–1499 https://doi.org/10.5713/ajas.15.0849
Zhang H, Gao S, Lercher MJ, Hu S, Chen WH (2012) EvoView, an online tool for visualizing, annotating and managing phylogenetic trees. Nucleic Acids Res 40:670–672 https://doi.org/10.1111/j.1757-2285.2011.08863.x
Zhu C, Sun B, Liu T, Zheng H, Gu W, He W, Sun F, Wang Y, Yang M, Bei W, Peng X, She Q, Xie L, Chen L (2017) Genomic and transcriptomic analyses reveal distinct biological functions for cold shock proteins (VpaCSP1a and VpaCSP1b) in Vibrio parahaemolyticus CHN25 during low-temperature survival. BMC Genomics 18:436 https://doi.org/10.1186/s12864-017-3784-5
Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions