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# In vitro probiotic characteristics of *Lactobacillus plantarum* ZDY 2013 and its modulatory effect on gut microbiota of mice

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## ABSTRACT

*Lactobacillus plantarum* ZDY 2013, a novel strain isolated from Chinese traditional fermented acid beans, was systematically evaluated for its survival capacity under stress conditions (pH, bile salt, simulated gastrointestinal tract, and antibiotics), production of exopolysaccharide and antagonism against 8 pathogens. Its effect on mice gut microbiota was also investigated by quantitative PCR and PCR-denaturing gradient gel electrophoresis. The results showed that ZDY 2013 can grow at pH 3.5 and survive at pH 2.0 for 6 h and at 0.45% bile salt for 3 h. The exopolysaccharide yield was up to 204 ± 7.68 mg/L. The survival rate of ZDY 2013 in a simulated gastrointestinal tract was as high as 65.84%. Antagonism test with a supernatant of ZDY 2013 showed maximum halo of 28 mm against *Listeria monocytogenes*. The inhibition order was as follows: *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Enterobacter sakazakii*, and *Staphylococcus aureus*. *Lactobacillus plantarum* ZDY 2013 was sensitive to some antibiotics (e.g., macrolide, sulfonamides, aminoglycoside, tetracyclines and β-lactams), whereas it was resistant to glycopeptides, quinolones, and cephalosporins antibiotics. Denaturing gradient gel electrophoresis profile demonstrated that ZDY 2013 administration altered the composition of the microbiota at various intestinal loci of the mice. Moreover, the quantitative PCR test showed that the administration of ZDY 2013 enhanced the populations of *Bifidobacterium* and *Lactobacillus* in either the colon or cecum, and reduced the potential enteropathogenic bacteria (e.g., *Enterococcus*, *Enterobacterium*, and *Clostridium perfringens*). *Lactobacillus plantarum* ZDY 2013 exhibited high resistance against low pH, bile salt, and gastrointestinal fluid, and possessed antibacterial and gut microbiota modulation properties with a potential application in the development of dairy food and nutraceuticals.
**Key words:** *Lactobacillus plantarum* ZDY 2013, stress resistance, quantitative PCR, antagonistics

## INTRODUCTION

The beneficial effects of probiotics (e.g., *Lactobacillus* and *Bifidobacterium*) have been documented by numerous investigations over many years, and mainly included improvement in the health of intestinal tract, enhancement of immune system, reduction of intestinal disorders, and risk of certain cancers (Salminen et al., 1998; Parvez et al., 2006; Jankovic et al., 2010). Generally, it has been assumed that probiotics, being of intestinal origin, can be used for human or animal consumption for exerting beneficial effects on host health through the interaction with host and for providing positive influence in the alleviation or prevention of diverse intestinal disorders (Borriello et al., 2003). For example, *Lactobacillus rhamnosus* GG, one of the most successful commercial *Lactobacillus* strains isolated from human gastrointestinal tract, has been used for nutraceutical supplement in numerous microecological studies (García-Ródenas et al., 2006).

In addition to isolating probiotics organisms from human feces, traditional fermented foods have been another important source for isolating probiotic organisms in many countries (ben Omar and Ampe, 2000; Tamime, 2002; Cheng et al., 2014). Isolation of probiotic organisms and evaluation of their properties has attracted a lot of attention in recent decades. In this context, isolation of *Lactobacillus plantarum* is important, as it is found in traditional fermented food, especially in fermented foods from Korea, China, and India (Salminen et al., 1998; Jankovic et al., 2010). Kleerebezem et al. (2003) carried out the complete genome sequence *L. plantarum* WCFS1, which was isolated from kimchi, and annotated the interaction of this organism with its environment at molecular level. However, most of the work on *L. plantarum* focused on its antioxidant,

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antimutagen, modulation of immune system, lowering cholesterol level, and reducing lactose-intolerance capabilities (Naidu et al., 1999; Fooks and Gibson, 2002; Gill, 2004; Parracho et al., 2007). The behavior of *L. plantarum* is diverse depending on different sources of isolation. Some isolates developed resistance to extreme acidic conditions, whereas others can survive in high concentration of bile salt (Liong and Shah, 2005; Guidone et al., 2014). Kaushik et al. (2009) reported that *L. plantarum* LP9 survived at pH 1.5 to 2.0 or in 1.5 to 2.0% oxgall bile for 2 h. Similarly, Lee et al. (2011) reported that *L. plantarum* CJLP133, isolated from kimchi, could survive at pH 2.5 for 3 h and in 0.3% oxgall for 24 h. Therefore, characterization of isolates of *L. plantarum* from traditional fermented food is important to ascertain the mechanism of probiotic action for promoting human health. Very limited information exists on in vivo interaction of *L. plantarum* with intestinal microbiota. In our study, a novel strain, *L. plantarum* ZDY 2013, was isolated from Chinese home-made acid beans and was systematically assessed for its survival in stress conditions (pH, bile salt, mimic gastrointestinal tract, and antibiotics) and in vivo interaction with indigenous microbial biota using quantitative real-time PCR (**qPCR**) combined with denaturing gradient gel electrophoresis (**DGGE**).

## MATERIALS AND METHODS

### Bacterial Strains Isolation and Identification

Traditional acid beans were collected from a village in Fujian province, China, and fermented under natural conditions without any additional bacteria. The bacterial strains were isolated from acid beans as described herein. Acid beans were collected from local village in Fujian province, China. Bacteria were isolated from acid beans according to a modified method described before (Wei et al., 2006; Wu et al., 2009). Briefly, 10 g of fermentation broth and beans were added to 90 mL of sterile PBS buffer, followed by homogenization. The suspension was serially diluted ($10^{-1}$, $10^{-2}$, $10^{-3}$) and 0.1 mL of each dilution was surface plated in duplicates on de Man, Rogosa, Sharpe (**MRS**) agar. The plates were incubated under anaerobic conditions at 37°C for 48 h. Colonies with distinct morphological differences, such as color, shape, and size, were selected and purified by streaking 3 times on MRS agar and were kept in MRS-agar stabs at 4°C, and frozen in 30% glycerol solution and stored at −80°C. A representative isolate was amplified by PCR method using the 16S rRNA universal primer. The amplicons were sequenced by Sangon Biotech, Ltd. (Shanghai, China) and the sequences were searched against NCBI database (http://www.ncbi.nlm.nih.gov/index.html) using the BLASTN algorithm. Based on these, the organism was classified as *L. plantarum* and was given the strain no. ZDY 2013.

### Acid and Bile Salt Tolerance Properties

The isolate *L. plantarum* ZDY 2013 was grown in sterile MRS broth at 37°C for 24 h, and centrifuged at 7,500 × *g* for 10 min at 4°C. The cell pellet was diluted 100× in sterile MRS broth and the pH was adjusted to 6.5, 5.0, 4.0, and 3.5 for acid adaptation; another set of media was adjusted to 3.0 and 2.0 for acid challenge studies. Bile salt tolerance was performed in the medium supplemented with 0, 0.15, 0.30, and 0.45% (wt/vol) bile salts (Yuanye Bio-Technology Ltd., Shanghai, China). Freshly prepared cultures were incubated at 37°C. The absorbance at 600 nm and pH values of fermentation cultures were measured immediately and at every 2 h. Viable counts were enumerated after acid challenge and bile salt tolerance test at 0, 3, 6, and 12 h.

### Exopolysaccharide Yield

The MRS broth (100 mL) was inoculated with 1% ZDY 2013 and cultured at 37°C in 250-mL Erlenmeyer flasks. Aliquots (5 mL) were withdrawn at various time points to determine the viable cell counts and exopolysaccharide (**EPS**) yield. The EPS from cell-free culture supernatant was extracted and detected by the methods as described by Li et al. (2014a). Briefly, bacterial cells were removed by centrifugation at 4,000 × *g* for 10 min at 4°C, and the cell-free culture supernatant was treated with ethanol overnight at 4°C followed by centrifugation (8,000 × *g*, 20 min, 4°C). The precipitate was dissolved in 5 mL of distilled water. Proteins were removed using Sevage agent (chloroform: n-butanol; 4:1) followed by centrifugation (8,000 × *g*, 10 min, 4°C). The supernatant was removed to another receptacle and precipitated with ethanol again. The precipitated EPS was dissolved with distilled water; EPS concentration was determined by the phenol-sulfuric acid assay method (Li et al., 2014a).

### Antimicrobial Activities of ZDY 2013

*Enterobacter sakazakii* ATCC 29544, *Escherichia coli* O 157*:* H 7, *Salmonella typhimurium* ATCC 13311, *Shigella sonnei* ATCC 25931, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* MCC 10104, *Listeria monocytogenes* CMCC 54007, and *Staphylococcus aureus* CMCC 26003 were selected to test the antibacterial

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