Global Advanced Research Journal of Food Science and Technology (ISSN: 2315-5098) Vol. 4(1) pp. 001-009, January 2015
Available online http://garj.org/garjfst/index.htm
Copyright © 2015 Global Advanced Research Journals

Full Length Research Paper

Adhesion and probiotic properties of *Lactobacillus plantarum* isolated from Chinese traditional fermented soybean paste

Qing Li[1,2+], Xiaoli Liu[1 2+], Mingsheng Dong[2], Jianzhong Zhou[1*], Ying Wang[1]

[1]Institution of Argo-product Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China
[2]College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China
[+]These authors contributed to the paper equally.
*Corresponding author, Jianzhong Zhou, Institution of Argo-product Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China, Tel: +86-25-84391571, E-mail: zjzluck@126.com

Accepted 04 January 2014

One *Lactobacillus* strain DJ-04 isolated from Chinese traditional fermented soybean paste was examined for its probiotic potential. The strain, identified as *Lactobacillus plantarum,* had great tolerance to stimulated gastrointestinal juices, 0.03-0.2% bile and 2-10% salt, and showed a strong antimicrobial activity against four pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp. and *Shigella* sp. In addition, good adhesion of *L. plantarum* DJ-04 to Caco-2 cells was observed in the study. Meanwhile, *L. plantarum* DJ-04 could significantly inhibited the adhesion of pathogenic bacteria to Caco-2 cells, with the inhibition percentage from 52.37% to 90.33%. These results suggested that *L. plantarum* DJ-04 could be a potential probiotic candidate in applications of fermented foods.

Keywords: *Lactobacillus plantarum*, Antimicrobial activity, Survival ability, Adhesion

INTRODUCTION

Lactic acid bacteria (LAB) are normal flora in the human gastrointestinal and urogenital tracts, conferring beneficial effects on the health of the host (Collado et al., 2004), such as inhibition on the invasion of pathogens, improvement of the epithelial barrier function and so on (Saxelin et al., 2005). In the past three decades, probiotics for beneficial gastrointestinal use had aroused great interest among different countries. Some strains of *Lactobacillus* (Lee et al., 2011; Ramos et al., 2013), *Bifidobacterium* (O'Mahony et al., 2005; Weizman et al., 2005; Xiao et al., 2003), *Enterococcuss* (Reid and Bruce, 2006), *Escherichia* (Tromm et al., 2004) have been explored as probiotics due to the growing evidence of their health benefits (Alvarez-Olmos and Oberhelman, 2001), and obviously, these strains have favorable general aspects (origin, safety, acid and bile tolerance and identity), technical aspects (growth properties in vitro and during processing) and functional and beneficial features (Iqbal et al., 2014).

Various traditional fermented foods are made by the local people in many regions throughout China. As their unique fermentation patterns, these products could be a meritorious source of native lactic acid bacteria. In order to regulate the gastrointestinal tract and contribute healthy effect to the host, probiotic bacteria should survive through the human gastrointestinal tract and colonize on the intestinal tract. The antimicrobial activity is also an important characteristic for the selection of probiotic bacteria, especially the competitive inhibition of the invasion and adhesion of pathogens to human intestinal epithelial cells.

Traditional fermented soybean paste with high salt content is very popular as side dishes or mating dishes in China. Lactic acid bacteria isolated from such high-salt environment could be better candidate for the fermentation of high-salt food, such as pickled vegetables, sausages, seafood sauce and so on. In the previous study, we obtained one *Lactobacillus* strain from traditional fermented soybean paste. The main objective of this study is to evaluate its survival activity in high-salt and imitative gastroenteric environments and its probiotic properties, including the antimicrobial ability, adhesion ability to Caco-2 cells and its competition-based adhesion with pathogens.

MATERIALS AND METHODS

The strain

The strain DJ-04 used in this study was isolated from Chinese traditional fermented soybean paste. The bean paste was collected from local families at Anshun County, Guizhou Province, located in the southeast China. The whole soybeans naturally fermented with high salt content and various spices after boiling and wilting. Five grams of paste were homogenized with 45 ml of 0.75% (w/v) sterile saline in a 150 ml flask for 30 seconds and serially diluted. Two hundred microliters of properly diluted sample were spread on MRS (Difco, USA) agar plates for isolation. The *Lactobacillus* strain isolated was cultured in MRS broth at 37°C for 20 h. Based on its 16s RNA sequence analysis and microscopic morphology, the strain DJ-04 was identified as *Lactobacillus plantarum.* The stock culture was stored in 20% glycerol at -80°C.

Tolerance and survival assay

The viability and survival of *L. plantarum* DJ-04 were evaluated in simulated gastric juice, simulated intestinal juice, bile solution and NaCl solution, respectively.

Simulated gastric juice was prepared according to the method described by Lucía (Abadia-Garcia et al., 2013) with some modifications. Simulated gastric juice was prepared by suspending pepsin (from porcine gastric mucosa, 250 units per mg, EC 232-629-3, Sigma Chemical Co., St. Louis, MO, USA) in a sterile NaCl solution (0.5%) with the final concentration of 3 g/L and adjusting the pH to 1.5, 2.5, 3.5, 4.5 with 1.0 M HCl, and

Table 1 The antimicrobial activity of *L. plantarum* DJ-04 against pathogens

	Lactobacillus plantarum DJ-04	
	supernatant(mm)	supernatant treated(mm)
Staphylococcus aureus	14.12±1.24	10.14±0.96
Salmonella sp.	13.06±0.68	12.08±0.24
Escherichia coli	12.24±0.42	9.88±0.62
Shigella sp.	11.46±1.02	10.06±0.78

Supernatant: cell-free supernatant. Supernatant treated: cell-free supernatant adjusted to pH 6.0 and treated with catalase.

Table 2 Inhibition of pathogens adhesion to Caco-2 cells by *L. plantarum* DJ-04

	Control	Co-cultured with DJ-04	Inhibition rate (%)
Staphylococcus aureus	132.63±10.22	12.83±4.06	90.23±2.01
Salmonella sp.	95.26±8.09	16.98±6.23	82.49±5.02
Escherichia coli	104.84±11.98	38.11±10.87	62.52±14.74
Shigella sp.	84.66±6.23	40.32±9.14	51.67±14.39

Note: Results were shown as values± SD.

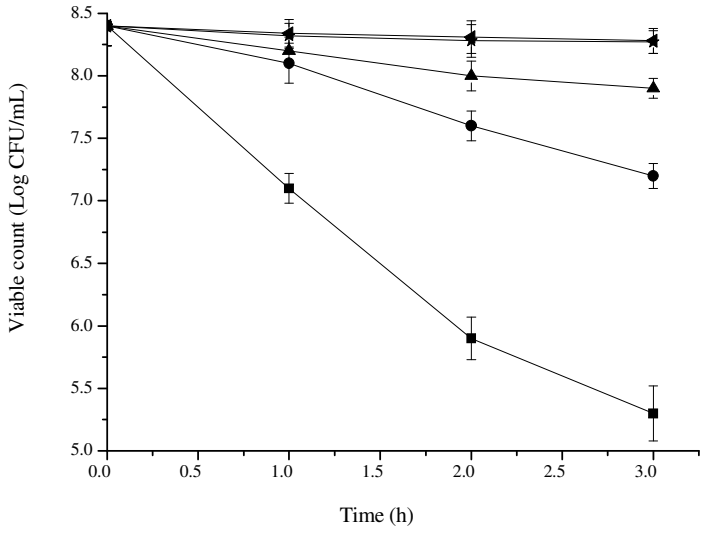

Figure 1 Survival of *L. plantarum* DJ-04 in stimulated gastrointestinal juices.
(■) pH1.5 , (◆) pH2.5, (▲) pH3.5, (★) pH4.5, (▼) Simulated intestinal juice.

then sterilized through 0.22 μm membrane (Millipore ,USA).

The imitative intestinal juice was prepared by dissolving bile salts in intestinal solution (6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L $CaCl_2$ and 1.386 g/L $NaHCO_3$, pH 7.5) to final concentration of 3.0 g/L and sterilized through 0.22 μm membrane (Chavarri et al., 2010) .

MRS broth was supplemented with cow bile at the final concentration of 0, 0.03, 0.1, 0.2, 0.3% (w/v) respectively, and then sterilized at 121°C for 20 min (Liu et al., 2013), in

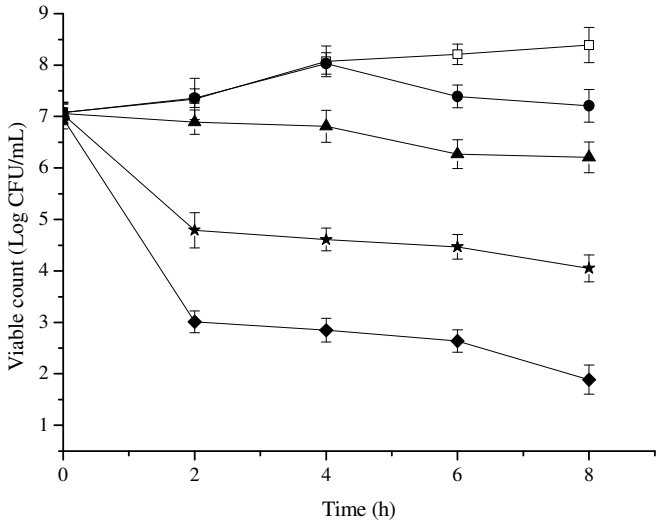

Figure 2 Growth of *L. plantarum* DJ-04 in MRS with different concentration of bile. (□) 0%, control group, (●) 0.03% , (▲) 0.1%, (★) 0.2% ,(◆) 0.3%.

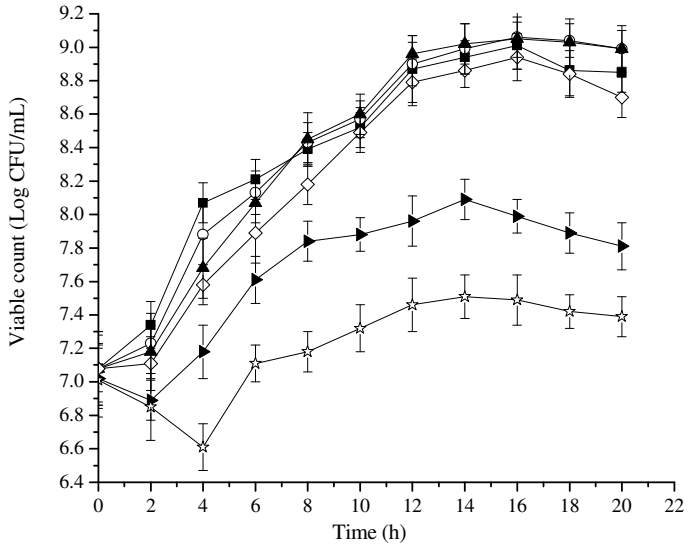

Figure 3 Growth of *L. plantarum* DJ-04 in MRS with different NaCl concern trations. (■) 0%, control group, (○) 2% ,(▲) 4% ,(◇) 6%, (►) 8% ,(☆) 10%.

order to evaluate the tolerance of *L. plantarum* DJ-04 to bile.

MRS broth with different concentrations of NaCl, 0, 2, 4, 6, 8 and 10% (w/v) were used to determine the

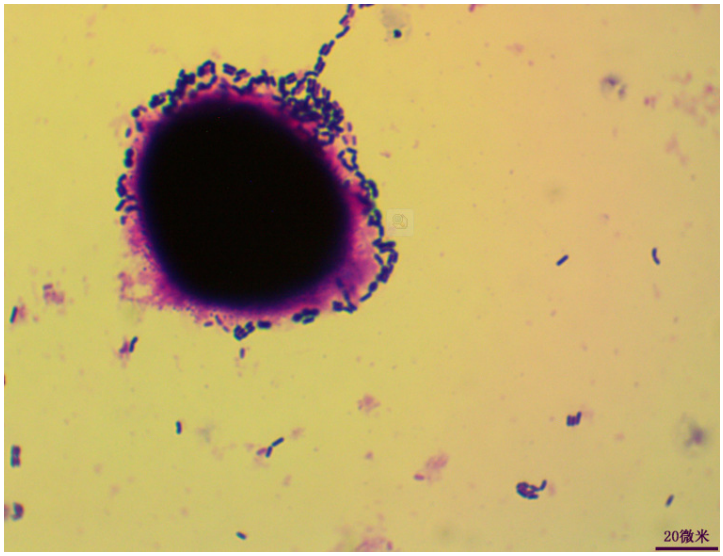

Figure 4. Adhesion to Caco-2 cells of *L. plantarum* DJ-04. Scale bar 20 µm.

tolerance of *L. plantarum* DJ-04 to salt (Pan et al., 2009).

In order to evaluate the survival viability of *L. plantarum* DJ-04 to the solutions described above, an inoculum of 2% (v/v) bacteria was added to the above solutions respectively and incubated at 37°C. Samples were taken at different times for microbial plate counting.

Antimicrobial activity assay

The agar well diffusion method was used to measure the inhibitory effect of *L. plantarum* DJ-04 on pathogenic bacteria (Ghanbari et al., 2013). Four pathogenic bacterial strains, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp. and *Shigella* sp. were provided by Nanjing Institute of Supervision & Testing on Product Quality (Jiangsu, China). Luria-Bertani (LB) agar containing 10^6 CFU/ml pathogenic bacteria was poured into 12-cm plates. Wells with 7 mm in diameter were made. After incubation at 37°C for 24 h, the culture of *L. plantarum* DJ-04 was centrifuged to get cell-free culture supernatant. The supernatant was devided into two portions. One was kept untreated, and the other portion was mixed with a sterile catalase solution (with the final concentration of 1000 U mL^{-1}, Sigma) after adjusting pH to 6.0, in order to eliminate the possible role of hydrogen peroxide and lactic acid. The original and treated supernatants were filled into the agar well separately. MRS medium alone was used as the negative control in each plate. The plates were then incubated at 37°C for 24-48 h. The diameters of the inhibition zone around the wells were measured. Each test was carried out in duplicate.

Cell lines and adhesion assay

The human colon adenocarcinoma Caco-2 cell lines were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Caco-2 cells were grown in Dulbecco's modified Eagle's medium (DMEM; HyClone, Laboratories Inc., Logan, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone) and antibiotics (100 U/ml penicillin, 100 mg/ml streptomycin; Gibco) in an incubator with 95% (v/v) humidified air and 5% (v/v) CO_2 at 37°C. The bacterial adhesion assay was carried out according to the method of Nueno-Palop and Narbad (2011) with some modifications. Caco-2 cells were seeded on 12-well tissue culture plates (Corning, Inc., Corning, NY, USA) at

a concentration of 2 × 10^5 cells per well and were cultured at 37°C in a humidified atmosphere of 5% CO_2 and 95% air until a confluent monolayer was obtained.

Caco-2 monolayers prepared in 12-well plates were washed twice with PBS (pH 7.2) to remove the excess free cells before the adhesion assay. The bacterial cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C and washed twice with PBS (pH 7.2), and then re-suspended in DMEM (antibiotic-free, fetal bovine serum-free). 1 ml of the bacteria suspension (1×10^9CFU/mL) was added to each well and then incubated at 37°C for different hours in 5% CO_2 atmosphere. Wells were washed 4 times with PBS (pH 7.2) to remove unattached bacterial cells. Then, 1 mL of 1% (v/v) Triton × 100 was added to corresponding well at 2 h. The suspension was then stirred for 10 min, detaching the cells from the well. Serial dilutions of the cell suspension were plated onto MRS agar (for lactic acid bacteria), Mannitol Salt agar (for Escherichia coli *Staphylococcus aureus*), and MAC agar (for *Salmonella* sp. and *Shigella* sp. growth) plates and incubated at 37 °C for 48 h to determine the viable bacterial cells.

The competition assay was carried out to access the effect of *L. planturam* DJ-04 on the attachment of pathogens to enterocyte-like cells (Xue et al., 2013). Caco-2 monolayers and bacterial suspension were prepared as above. The mixture of 500µL pathogen suspension and 500µL *Lactobacillus* suspension were injected into the wells. 500µL PBS instead of *Lactobacillus* suspension was used as the control. After incubated at 37°C for 2 h in 5% CO_2 atmosphere, monolayers was washed 4 times with PBS and afterwards the cells were lysed with 1% (v/v) Triton × 100. Serial dilutions of the mixtures were then plated onto the Mannitol Salt agar (for *Staphylococcus aureus* growth), and MAC agar (for *Salmonella* sp. and *Shigella* sp.) plates, separately, and incubated at 37 °C for 48 h to determine the viable bacterial cell number.

Statistical analysis

All the experiments were carried out three times independently. The data are presented as the average of the three determinations. Paired t tests and analysis of variance (ANOVA) were carried out with SPSS 11.0 software.

RESULTS AND DISCUSSION

Evaluation of *L. plantarum* DJ-04 to simulated human gut challenges

The ability of DJ-04 to cope with the stressful human gut conditions was evaluated by exposing the cells through simulated gastrointestinal tract environmental conditions. The viability of *L. plantarum* DJ-04 after exposure to the simulated gastrointestinal juices with different pH was shown in Figure 1. The harsh low pH condition decreased survival rate of DJ-04 greatly. At higher pH conditions, the resistance of *L. plantarum* DJ-04 increased rapidly. Viability of DJ-04 upon pH 4.5 and 3.5 for 3h was 74.13% and 31.62% respectively, relative to unexposed original counting, considerably higher than those results of 0.08% and 6.31% for lower pH 1.5 and 2.5 (Figure 1). In order to exert the beneficial effects in human intestinal tract, *Lactobacillus* should resist the stressful gastrointestinal conditions and keep certain transit tolerance. pH value in human stomach ranges from 1.5 to 4.5 (Bao et al., 2010), and in this study, *L. plantarum* DJ-04 had good resistance to simulated gastric and intestinal juices, meeting the prerequisite of exerting potential probiotic functions.

The bile salt concentration in the human intestine different as time and segments of the intestine, the average concentration is believed to be about 0.3% (w/v) (Garcia-Hernandez et al., 2012). Results in Figure 2 show the tolerance of *L. plantarum* DJ-04 to bile. Like the control without bile salt, the viable cell counts in MRS broth with 0.03% bile increased in the first 4 hours, while the growth in MRS broth with higher bile salt contents was immediately inhibited since the beginning of the experiment. After 8 h incubation in MRS broth with 0.2% bile salt, the viable bacteria still reached about 10^5 CFU/ml, while only 10^3 CFU/ml left in MRS containing 0.3% bile.

The salt with high concentration can improve the flavour of the fermented food, and also inhibit the growth of

pathogenic bacteria. Mohd Adnan (2007) suggest that lactic acid bacteria strain can grow in a concentration of NaCl less than 10%, because bacteria cells would experience a loss of cell pressure, which would then affect the physiology, enzyme and metabolism of the cells. *L. plantarum* DJ-04 had a significant survival ability to salt ranging from 0% to 10% (Figure 3). Lower content of salt below 4%, seemed to stimulate the growth of DJ-04. Higher content of the salt above 8% had an inhibition effect on the growth of *L. plantarum* DJ-04, but the cells counts could still reach to 10^7 CFU/ml.

Antimicrobial activity

Antimicrobial activity is recognized as an important property for functional probiotics. Many papers have reported that *Lactobacillus* spp. have antimicrobial activity due to different factors. Production of organic acids and bacteriocins are the main contributors to the antimicrobial activity (Liu et al., 2013; Makras et al., 2006; Pan et al., 2009; Zhang et al., 2011).The inhibition of *L. plantarum* DJ-04 against pathogen bacteria was shown in Table 1. The cell-free supernatant showed great antimicrobial activity to all the examined pathogen bacteria with the inhibition zone ranging from 12.62±1.14 mm to 14.47±1.56 mm. The treatment of adjusting pH to 6.0 and catalase weakened slightly the inhibition activity of the supernatant, by the percentage from 7.50% to 28.19%, indicating the possible production of bacteriocins in the metabolites of *L. plantarum* DJ-04.

Adhesion ability of *L. plantarum* DJ-04 to Caco-2 cells

Adhesion ability is significantly important of promoting probiotic bacteria to colonize the gastrointestinal tract and conferring a healthy benefit to the host. The adhesive ability to epithelial cells and mucosal surfaces has been evaluated to be an important property of many probiotic bacterial strains, resulting in the colonization to the gastrointestinal tract and consequently conferring health benefits to the host (Tuo et al., 2013). Caco-2 has been mostly used as a cellular model since it displays the characteristics of a mature enterocyte in vitro (Garc I A-Cayuela et al., 2014).The *L. plantarum* DJ-04 showed good adhesion ability to Caco-2 cells (Figure 4). After co-inhibited with Caco-2 cells for 2h, *L. plantarum* DJ-04 showed the greatest adhesion value of 78 ± 8.24 bacteria cells/Caco-2 cells (Figure 4). Many other studies showed that lactobacilli have a strong adherence to epithelial cells. Liu (2013) reported that the adherence of the *L. plantarum* CCFM 233 to epithelial cells was 45.33 ± 5.78.

Competitive effect of DJ-04 on the attachment of pathogens to Caco-2 cells

Pathogens, such as *Staphylococcus aureus*, *Salmonella* sp., etc., are responsible for a diverse spectrum of human and animal diseases, and increasing evidence demonstrated that adhesion of these pathogens to intestinal epithelial cells is a key process to survive and colonize the gastrointestinal tract and mucosal infection (Mempel et al., 1998; Tristan et al., 2003). Adherence to intestinal epithelial cells could bring adverse effects such as resulting in excessive growth of intestinal pathogens during antibiotic treatment and leading to antibiotic-associated diarrhea (Ackermann et al., 2005; Boyce and Havill, 2005). Inhibition of the adhesion and invasion of pathogens into epithelial cells is the first step in disease prevention. Competition assay was carried out to evaluate the role of *L. plantarum* DJ-04 on the adhesion of four pathogens, *Staphylococcus aureus*, Salmonella sp., Escherichia coli and *Shigella* sp. to Caco-2 cells. The results of *L. plantarum* DJ-04 inhibited the adhesion of pathogens to Caco-2 cells were shown in Table 2. All the adherent pathogens were obviously reduced when co-incubated with *L. plantarum* DJ-04. The reduction of *Staphylococcus aureus* and *Salmonella* sp. were more significant than the other two pathogens, with the percentage of 90.33% and 82.18% respectively.

CONCLUSIONS

The results displayed in this study demonstrated that *L. plantarum* DJ-04 from Chinese traditional fermented soybean paste presented interesting probiotic characteristics, like significant tolerance to gastric juices and salt concentration, as well as good antimicrobial activity, adhesion to Caco-2 cells and inhibition activity against pathogens adhesion to Caco-2 cells. The strain could survive greatly through simulated gastrointestinal tract environment with harsh low pH conditions, and about 0.3% bile salt. Moderate NaCl concentrate below 4% stimulated the growth of DJ-04, and higher salt content had no significant inhibitive effect on the growth. The considerable viability of *L. plantarum* DJ-04 to stressful conditions may be of benefit to the food industry. Good inhibitive activity against pathogenic bacteria and adhesive ability to Caco-2 cell were also observed for *L. plantarum* DJ-04. In addition, when co-incubated with pathogens, *L. plantarum* DJ-04 could compete with pathogens to reduce significantly the attachment of pathogens to Caco-2 cells, so that weaken greatly the colonization of pathogens to epithelial cells. These results indicated that *L. plantarum* DJ-04 can be a potential candidate with probiotic properties. Further research would be carried out to study the mechanism of the competitive attachment to attachment to Caco-2 cells between *L. plantarum* and pathogens.

Acknowledgement

The study was supported by grant from Jiangsu Agricultural Technological Innovation Fundation SCX(13)3192.

REFERENCES

Abadia-Garcia L, Cardador A, Martin Del Campo ST, Arvizu SM, Castano-Tostado E，et al. (2013). Influence of probiotic strains added to cottage cheese on generation of potentially antioxidant peptides, anti-listerial activity, and survival of probiotic microorganisms in simulated gastrointestinal conditions. Int Dairy J. 33:191-197.

Ackermann G, Thomalla S, Ackermann F, Schaumann R, Rodloff AC and Ruf BR. (2005). Prevalence and characteristics of bacteria and host factors in an outbreak situation of antibiotic-associated diarrhoea. J Med Microbiol. 54:149-153.

Alvarez-Olmos MI and Oberhelman RA. (2001). Probiotic agents and infectious diseases: A modern perspective on a traditional therapy. Clin Infect Dis. 32:1567-1576.

Bao Y, Zhang Y, Zhang Y, Liu Y, Wang S, Dong X, Wang Y and Zhang H. (2010). Screening of potential probiotic properties of Lactobacillus fermentum isolated from traditional dairy products. Food Control 21:695-701.

Boyce JM and Havill NL. (2005). Nosocomial antibiotic-associated diarrhea associated with enterotoxin-producing strains of methicillin-resistant Staphylococcus aureus. Am J Gastroenterol . 100:1828-1834.

Chavarri M, Maranon I, Ares R, Ibanez FC, Marzo F and Del Carmen Villaran M. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. Int J Food Microbiol. 142:185-189.

Collado MC, Meriluoto J, Salminen S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains. Eur Food Res Technol. 226:1065-1073

Garc I A-Cayuela TAS, Korany AM, Bustos I, de Cadi N Anos LPGO, Requena T, Pel A Ez C and Mart I Nez-Cuesta MC. (2014). Adhesion Abilities of Dairy Lactobacillus Plantarum Strains Showing an Aggregation Phenotype. Food Res Int. 57:44-50.

Garcia-Hernandez Y, Rodriguez Z, Brandao LR, Rosa CA, Nicoli JR, Elias Iglesias A, et al. (2012). Identification and in vitro screening of avian yeasts for use as probiotic. Res Vet Sci. 93:798-802.

Ghanbari M, Jami M, Kneifel W and Domig KJ. (2013). Antimicrobial activity and partial characterization of bacteriocins produced by lactobacilli isolated from Sturgeon fish. Food Control 32:379-385.

Iqbal MZ, Qadir MI, Hussain T, Janbaz KH, Khan YH and Ahmad B. (2014). Probiotics and their beneficial effects against various diseases. Pakistan Journal of Pharmaceutical Sciences 27:405-415.

Lee J, Yun HS, Cho KW, Oh S, Kim SH, Chun T, Kim B and Whang KY. (2011). Evaluation of probiotic characteristics of newly isolated Lactobacillus spp.: Immune modulation and longevity. Int J Food Microbiol. 148:80-86.

Liu X, Liu W, Zhang Q, Tian F, Wang G, Zhang H and Chen W. (2013).

Screening of lactobacilli with antagonistic activity against enteroinvasive Escherichia coli. Food Control 30:563-568.

Makras L, Triantafyllou V, Fayol-Messaoudi D, Adriany T, Zoumpopoulou G, Tsakalidou E, Servin A and De Vuyst L. (2006). Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards Salmonella enterica serovar Typhimurium reveals a role for lactic acid and other inhibitory compounds. Res Microbiol. 157:241-247.

Mempel M, Schmidt T, Weidinger S, Schnopp C, Foster T, Ring J and Abeck D. (1998). Role of Staphylococcus aureus surface-associated proteins in the attachment to cultured HaCaT keratinocytes in a new adhesion assay. J Invest Dermatol. 111:452-456.

Mohd Adnan AF and Tan IKP. (2007). Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. Bioresource Technol. 98:1380-1385.

Nueno-Palop C and Narbad A. (2011). Probiotic assessment of Enterococcus faecalis CP58 isolated from human gut. Int J Food Microbiol. 145:390-394.

O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo FY, Chen KS, et al. (2005). Lactobacillus and Bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. Gastroenterology 128:541-551.

Pan X, Chen F, Wu T, Tang H and Zhao Z. (2009). The acid, bile tolerance and antimicrobial property of Lactobacillus acidophilus NIT. Food Control 20:598-602.

Ramos CL, Thorsen L, Schwan RF and Jespersen L. (2013). Strain-specific probiotics properties of Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus brevis isolates from Brazilian food products. Food Microbiol. 36:22-29.

Reid G and Bruce AW. (2006). Probiotics to prevent urinary tract infections: the rationale and evidence. World J Urol. 24:28-32.

Saxelin M, Tynkkynen S, Mattila-Sandholm T and de Vos WM. (2005). Probiotic and other functional microbes: from markets to mechanisms. Curr Opin Biotech. 16: 204-211.

Tristan A, Ying L, Bes M, Etienne J, Vandenesch F and Lina G. (2003). Use of multiplex PCR to identify Staphylococcus aureus adhesins involved in human hematogenous infections. J Clin Microbiol. 41:4465-4467.

Tromm A, Niewerth U, Khoury M, Baestlein E, Wilhelms G, Schulze J and Stolte M. (2004). The probiotic E. coli strain Nissle 1917 for the treatment of collagenous colitis: First results of an open-label trial. Z Gastroenterol. 42:365-369.

Tuo Y, Yu H, Ai L, Wu Z, Guo B and Chen W. (2013). Aggregation and adhesion properties of 22 Lactobacillus strains. J Dairy Sci. 96:4252-4257.

Weizman Z, Asli G and Alsheikh A. (2005). Effect of a probiotic infant formula on infections in child care centers: Comparison of two probiotic agents. Pediatrics 115:5-9.

Xiao JZ, Kondo S, Takahashi N, Miyaji K, Oshida K, Hiramatsu A, et al. (2003). Effects of milk products fermented by Bifidobacterium longum on blood lipids in rats and healthy adult male volunteers. J Dairy Sci. 86:2452-2461.

Xue C, Zhang L, Li H, Wang S, Li Q, Luo X, et al. (2013). Functionality of the S-layer proteins from Lactobacillus in the competitive against enteropathogens infection. Eur Food Res Technol. 236:249-255.

Zhang Y, Zhang L, Du M, Yi H, Guo C, Tuo Y, et al. (2011). Antimicrobial activity against Shigella sonnei and probiotic properties of wild lactobacilli from fermented food. Microbiol Res. 167:27-31.