Kasetsart J. (Nat. Sci.) 45 : 1071 - 1082 (2011)

Lactobacillus plantarum Strains from Fermented Vegetables as Potential Probiotics

Wanticha Lapsiri, Sunee Nitisinprasert and Penkhae Wanchaitanawong*

ABSTRACT

The present study was conducted in order to evaluate the probiotic properties of 13 strains of *Lactobacillus plantarum.* All strains exhibited adhesion ability by autoaggregation and cell hydrophobicity determination. Six strains succeeded in exhibiting positive coaggregation with *Escherichia coli* O157:H7 DMST 12743 and *Salmonella* Typhimurium ATCC 13311. All strains were resistant to clindamycin, kanamycin, ciprofloxacin, streptomycin, cepfoxitin, oxacillin and vancomycin but susceptible to chloramphenicol, rifampin and penicillin. Most strains exhibited antimicrobial activity against the pathogens by the spot-on-lawn method. Seven strains could display moderate acid tolerance and high bile tolerance. Moreover, four strains showed heat tolerance, with survival rates exceeding 80% at 65 °C for 30 min. Based on the probiotic criteria, *L. plantarum* TISTR 2075 had good potential as a probiotic for further food applications.

Keywords: *Lactobacillus plantarum*, aggregation, cell surface hydrophobicity, antimicrobial activity, gastrointestinal tract tolerance

INTRODUCTION

Lactobacillus plantarum is one of the lactic-acid-producing bacteria which have been used for centuries in human food preservation. The strain is a non-pathogenic, Gram-positive bacterium naturally existing in human saliva and the gastrointestinal tract (de Vries *et al*., 2006; Michida *et al*., 2006). It is commonly found in fermented vegetable food products and considered as a GRAS (generally recognized as safe) microorganism for human consumption (Cebeci and Gürakan, 2003; Brinques and Ayub, 2011). In recent years, several studies have focused on *L. plantarum* as a probiotic because there is increasing consumer demand for nondairy-based probiotic products. Furthermore, lactose intolerance and the cholesterol content are two major drawbacks related to fermented dairy products (Prado *et al*., 2008). In addition, *L. plantarum* has proven to exert a range of health promoting activities such as lowering cholesterol, reducing pain and reducing constipation associated with irritable bowel syndrome (Candela *et al.*, 2008; Sirilun *et al.*, 2010). Currently, only a few strains of *L. plantarum* are commercially available for probiotic application such as *L. plantarum* 299v and *L. plantarum* Lp01. Many clinical studies have proven that such strains have potential probiotic properties in the human intestinal tract (Cebeci and Gürakan, 2003; Giraffa *et al.*, 2010).

Probiotics are defined by FAO/WHO (2002) as "live microorganisms which, when administered in adequate amounts, confer

Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fagipkw@ku.ac.th

Received date : 06/05/11 Accepted date : 08/08/11

health benefit on the host". To achieve health benefits, bacteria must be viable and available at high concentrations of at least 1×10^6 CFU. mL^{-1} or 1×10^6 CFU.g^{-1} of product at the time of consumption (Kailasapathy and Chin, 2000). The criteria for selecting a good probiotic strain have been listed by several authors and include: being of human origin, a lack of pathogenicity, survival during gastric transit, tolerance to bile salt, adherence to the gut epithelial tissue, competitive exclusion of pathogens and antibiotic resistance (de Vries *et al.*, 2006; Giraffa *et al.*, 2010). Furthermore, several technological aspects have to be considered in the selection of a probiotic including viability during processing and stability in production and during storage (Kosin and Rakshit, 2006). These properties make it possible to screen and select specific probiotic strains for both food and technological uses. Therefore, the aim of the present study was to attempt to find new strains of nondairy probiotics from fermented vegetables, with any new strain carrying the probiotic traits mentioned above so that it has a favorable health effect on humans.

MATERIALS AND METHODS

Microorganisms

Thirteen strains of *L. plantarum* isolated from fermented vegetables were obtained from the Microbiological Resources Center (MIRCEN), Thailand Institute of Scientific and Technological Research (TISTR), Thailand. All test strains were preserved in de Man, Rogosa, Sharpe (MRS) broth (Merck, Darmstadt, Germany) with 20% (v/v) glycerol content at -20 °C. *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311 were purchased from the Department of Medical Science, Ministry of Public Health, Thailand. The indicator strains were grown in tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA) supplemented with 0.6% yeast extract (YE) at 37 °C. All microorganisms were subcultured twice and incubated at 37 °C for 24 hr for routine analysis.

Cell surface hydrophobicity assay

Cell surface hydrophobicity was determined by the method of Kos *et al*. (2003) with minor modifications. Overnight cultures of *L. plantarum* were harvested by centrifugation at 5,000×*g* for 15 min. The cell pellets were washed twice and resuspended in sterile 0.85% NaCl solution to give an optical density of 0.5 at 600 nm (OD_0). To test tubes containing 3 mL of washed cells, 1 mL of toluene or xylene (Panreac Quimica SAU, Barcelona, Spain) was added. The mixtures were vortexed for 90 s. After incubation at room temperature for 15 min, the aqueous phase was removed and its optical density at 600 nm (OD_1) was then measured. The percentage of cell surface hydrophobicity was calculated as $[1-(OD_1/OD_0)] \times 100$.

Autoaggregation assay

Autoaggregation assays were performed according to Del Re *et al*. (2000). Overnight cultures were harvested by centrifugation at 5,000×*g* for 15 min. The cell pellets were washed twice and resuspended in sterile phosphate buffer saline (PBS) to give viable cell counts of approximately 1×10^8 CFU.mL^{-1}. The cell suspensions (4 mL) were vortexed for 10 s. During incubation at room temperature for 5 hr, 0.1 mL of the upper suspensions was transferred to another tube containing 3.9 mL of PBS and the optical density (OD_t) was measured at 600 nm every 1 hr. The autoaggregation percentage was expressed as $[1 (OD_t/OD_0)] \times 100$, where OD_t represents the optical density at time t = 1, 2, 3, 4 and 5 h and OD_0 is the optical density at t = 0.

Coaggregation assay

Coaggregation between *L. plantarum* and *E. coli* O157:H7 DMST 12743 or *S.* Typhimurium ATCC 13311 was investigated. The cell suspensions

were prepared in the same manner as described in the autoaggregation assay. Equal volumes (2 mL) of *L. plantarum* and pathogen cell suspension were mixed together by vortexing for 10 s. Control tubes were set up at the same time, containing 4 mL of each bacterial suspension on its own. The optical density at 600 nm of the suspensions was measured after 5 hr of incubation at room temperature. Samples were taken using the same procedure as in the autoaggregation assay. The percentage of coaggregation was calculated using equation 1 (Handley *et al*., 1987):

$$Coaggregation\ (\%) = \frac{\left(\frac{OD_x + OD_y}{2}\right) - OD_{(x+y)}}{\left(\frac{OD_x + OD_y}{2}\right)} \times 100 \quad (1)$$

where x and y represent *L. plantarum* and the pathogen, respectively and $(x + y)$ represents the mixture of *L. plantarum* and each pathogen.

Antibiotic resistance assay

The antibiotic sensitivity of *L. plantarum* was determined by the Bauer-Kirby method (Bauer *et al*., 1966). The optical density at 600 nm of the overnight culture was adjusted to 0.08–0.1 (equivalent to 1–2 × 10^8 CFU.mL^{-1}). The inocula were spread evenly over the entire surface of the MRS agar plates. Subsequently, paper discs containing the antibiotics (BD BBL™, Becton Dickinson, MD, USA) were laid on the plates. After incubation at 37 °C for 24 hr, the inhibition zones were measured inclusive of the diameter of the discs. Results were expressed as sensitive, S (diameter ≥ 21 mm); intermediate sensitive, I (diameter 16–20 mm) and resistant, R (diameter ≤ 15 mm) after Vlková *et al*. (2006).

Antimicrobial activity assay

Cell-free supernatants (CFS) of overnight cultures of the 13 strains of *L. plantarum* were evaluated for antimicrobial activity by the spot-on-lawn method as described by Alemu *et al*. (2002). The pH of each CFS was not adjusted and adjusted to 5.0 and 6.0 with 5 M NaOH to eliminate the inhibitory effect of organic acid, and filter-sterilized with disposable bacterial filters (0.22 μm; Minisarts®, satorius stedim, Goettingen, Germany). A TSAYE plate (1.5% agar) was overlaid with 5 mL of soft TSAYE (0.75% agar) containing 10 μL of indicator strain (*E. coli* O157:H7 DMST 12743 or *S*. Typhimurium ATCC 13311) (approximately 1 × 10^6 CFU. mL^{-1}). Serially diluted CFS (10 μL) was spotted onto the indicator plates. The plates were then incubated at 37 °C for 6 hr. The inhibition zone was revealed by the formation of a clear zone in the indicator bacterial lawn. Antimicrobial activity was expressed in arbitrary units (AU) per milliliter of the original cultures. An arbitrary unit was defined as the reciprocal of the highest dilution which produces a clear zone of growth inhibition of the indicator strain calculated as (D × 1,000)/10, where D denotes the dilution factor.

Preparation of simulated gastric and small intestinal juices

Simulated gastric juice was prepared by means of suspension of pepsin (1:10,000; ICN, Sigma, Basingstoke, Hampshire, UK) in sterile 0.5% NaCl to a final concentration of 3 $g.L^{-1}$ and adjusted to pH 2.0 with concentrated HCl (Michida *et al*., 2006).

Simulated small intestinal juice was prepared by suspension of pancreatin USP (P1500, Sigma, Basingstoke, Hampshire, UK) in a sterile 0.5% NaCl to a final concentration of 1 $g.L^{-1}$ 0.45% bile salt content (Oxoid, Basingstoke, Hampshire, UK) and adjusted to pH 8.0 with sterile 0.1 $mol.L^{-1}$ NaOH (Huang and Adam, 2004).

Simulated gastrointestinal tract tolerance

An aliquot (0.2 mL) of each washed cell suspension was transferred to a sterile tube, mixed with 0.3 mL sterile 0.5% NaCl and finally

blended with 1.0 mL of simulated gastric juice (pH 2.0) or small intestinal juice (pH 8.0) in the presence of 0.45% bile salt. In the simulated gastric juice tolerance determination, viable cell counts were measured after 30, 60, 90 and 180 min. In the simulated small intestinal juice tolerance determination, viable cell counts were measured after 240 min.

Heat tolerance

Heat tolerance of *L. plantarum* was determined according to Ding and Shah (2007). Overnight cultures of *L. plantarum* were incubated at 65 °C. The cell viability was monitored at 0, 30 and 60 min.

Determination of viable cell counts

Viable cell counts were determined by the standard plate count method on MRS agar plate. The plates were incubated at 37 °C for 24 hr. The viable cell counts were expressed as $\log_{10}$ value. mL^{-1}. The percentage of cell survival was defined as follows: survival rate (%) = (log N/log N_0) × 100, where N represents the number of viable cells ($CFU.mL^{-1}$) after exposure and N_0 denotes the initial viable cell count ($CFU.mL^{-1}$) prior to exposure (Bao *et al*., 2010).

Statistical analysis

Each result was expressed as the mean ± SD of three determinations. The data were assessed using analysis of variance (ANOVA) with a level of significance at $P < 0.05$. Significant divergences among mean values were determined with Duncan's multiple range tests. All statistical analyses were performed using SPSS Software, version 12 (SPSS, now a part of IBM Corp.; White Plains, NY, USA).

RESULTS AND DISCUSSION

Aggregation and cell surface hydrophobicity

Aggregation and cell surface hydrophobicity were used as preliminary screens for the probiotic properties of the 13 strains of *L. plantarum*. As shown in Table 1, all test strains exhibited a strong autoaggregation of 63.80–86.97% after 5 hr incubation. Of the 13 strains, TISTR 2072, TISTR 2073, TISTR 2075, TISTR 2079, TISTR 2081and TISTR 2082 showed coaggregation ability with both *E. coli* O157:H7 DMST 12743 (3.68–18.75%) as shown in Figure 1 and with *S.* Typhimurium ATCC 13311 (3.85–12.16%), while TISTR 2078 only showed coaggregation ability with *E. coli* O157:H7 DMST

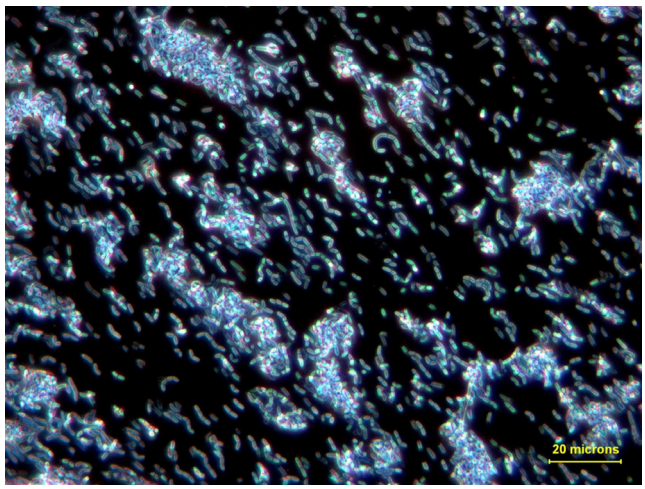

Figure 1 Coaggregation ability between *L. plantarum* TISTR 2072 and *E. coli* O157:H7 DMST 12743.

Table 1 Autoaggregation, coaggregation and cell surface hydrophobicity of 13 strains of *L. plantarum.*

Strains	Aggregation (% ± SD)							Cell surface hydrophobicity (% ± SD)	
	Autoaggregation (h)					Coaggregation (% ± SD)			
	1	2	3	4	5	with *E. coli*	with *S.* Typhimurium	Toluene	Xylene
TISTR 2070	7.25±2.37	39.67±3.26	22.55±5.80	56.52±2.65	73.82±0.62^{de}	N.D.	N.D.	9.03±3.30^{g}	2.47±1.78^{g}
TISTR 2071	0.56±0.24	50.46±3.16	30.94±0.77	56.79±0.50	63.80±1.35^{g}	N.D.	N.D.	5.93±5.74^{g}	1.53±0.84^{g}
TISTR 2072	1.33±1.00	48.00±1.63	54.17±2.89	75.67±1.28	86.97±1.96^{a}	18.75±8.44^{a}	3.85±3.14^{b}	86.46±2.90^{b}	52.63±10.29^{b}
TISTR 2073	1.61±1.08	47.18±2.75	54.97±0.78	68.82±2.48	73.27±8.71^{de}	3.68±1.47^{b}	3.91±1.56^{b}	67.26±4.11^{c}	48.76±3.25^{bc}
TISTR 2074	1.06±1.06	51.98±1.52	54.37±0.76	70.11±2.78	76.61±1.04^{bcd}	N.D.	N.D.	57.64±3.25^{d}	37.38±7.61^{d}
TISTR 2075	1.58±1.05	60.13±1.51	60.53±2.40	70.00±0.61	75.62±0.86^{cd}	9.26±2.14^{b}	10.00±5.16^{ab}	99.79±0.08^{a}	80.15±3.77^{a}
TISTR 2076	1.43±0.95	51.79±3.17	55.36±0.69	65.48±1.96	68.02±1.05^{f}	N.D.	N.D.	10.24±5.80^{g}	0.65±0.60^{g}
TISTR 2077	2.60±1.66	50.65±1.50	60.23±3.46	69.91±1.30	72.77±3.34^{de}	N.D.	N.D.	10.56±5.28^{g}	1.71±1.19^{g}
TISTR 2078	14.34±0.74	58.92±1.06	67.60±0.88	70.96±0.42	77.03±0.94^{bcd}	8.11±2.21^{b}	N.D.	9.76±8.23^{g}	5.60±1.54^{fg}
TISTR 2079	6.85±1.54	54.64±1.56	67.49±2.08	70.16±2.79	70.87±1.63^{ef}	8.97±3.31^{b}	12.16±6.43^{a}	55.92±4.97^{d}	43.37±1.23^{cd}
TISTR 2080	17.83±2.88	60.93±1.00	70.94±0.84	72.55±1.44	80.68±0.34^{b}	N.D.	N.D.	3.91±2.52^{g}	0.42±0.36^{g}
TISTR 2081	10.04±0.77	56.56±0.67	65.49±0.48	72.39±2.47	74.73±1.56^{de}	5.88±4.80^{b}	5.47±2.99^{ab}	47.14±1.87^{e}	43.03±2.68^{cd}
TISTR 2082	10.12±4.98	68.77±1.12	72.76±1.12	68.87±2.70	79.66±1.36^{bc}	9.03±3.50^{b}	10.29±7.78^{ab}	48.39±2.04^{e}	29.69±11.66^{e}
E. coli	2.89±2.48	33.37±4.34	67.98±0.55	70.66±1.07	73.48±3.46^{de}	-	-	23.27±0.69^{f}	27.54±6.80^{e}
S. Typhimurium	3.60±1.04	35.14±1.56	74.10±0.92	75.68±2.08	77.22±0.38^{bcd}	-	-	45.62±7.55^{e}	11.79±2.53^{f}

N.D. = not detected; - = not determined.

Values in the same column with different lower case letters (a–g) are significantly different by Duncan's multiple range test ($P < 0.05$).

12743 (8.11%). The coaggregation is thought to be linked to the ability to interact closely with undesirable bacteria representing competitive exclusion of the test strains against enteric pathogens (Taheri *et al.*, 2009a). Furthermore, cell surface hydrophobicity was determined using toluene and xylene. For all test strains, a significant difference of cell surface hydrophobicity was observed. Seven strains (TISTR 2072, TISTR 2073, TISTR 2074, TISTR 2075, TISTR 2079, TISTR 2081 and TISTR 2082) exhibited high cell surface hydrophobicity in toluene and xylene ranging from 47.14 to 99.79% and 29.69 to 80.15%, respectively, which was higher than that of *E. coli* O157:H7 DMST 12743 (23.27% and 27.54%) and *S.* Typhimurium ATCC 13311 (45.62% and 11.79%). This suggested that the ability of these strains to adhere to epithelial cells was greater than that of the pathogens (Garriga *et al.*, 1998; Taheri *et al.*, 2009b). However, other test strains showed lower cell surface hydrophobicity (3.91–10.56% in toluene and 0.42–5.60% in xylene). These differences in cell surface hydrophobicity could be due to variation in the level of expression of cell surface protein among strains of a species as well as due to environmental conditions which could affect the expression of surface protein (Kaushik *et al.*, 2009).

Antibiotic resistance

Antibiotic disc diffusion susceptibility of all test strains is summarized in Table 2. All strains were totally resistant to clindamycin, kanamycin, ciprofloxacin, streptomycin, cepfoxitin, oxacillin and vancomycin, whereas they were sensitive to chloramphenicol, rifampin and penicillin. Among antibiotic resistance, it is known that vancomycin resistance is of major concern because it is broadly efficacious against clinical infections caused by multidrug-resistant pathogens (Mathur and Singh, 2005; Zhou *et al.*, 2005). The result of all test strains showing resistance to vancomycin in the present study was in agreement with Herreros *et al*. (2005) and Zhou *et al*. (2005).

Antimicrobial activity of *L. plantarum*

As shown in Table 3, CFS (pH 3.65–3.78) obtained from all test strains displayed growth inhibition of *E. coli* O157:H7 DMST 12743 and *S*. Typhimurium ATCC 13311. Antimicrobial activity of CFS (pH 5.0) was observed against *E. coli* O157:H7 DMST 12743 (100–200 AU.mL^{-1}) and *S*. Typhimurium ATCC 13311 (100 AU.mL^{-1}). Only CFS (pH 6.0) of TISTR 2070, TISTR 2071, TISTR 2072, TISTR 2073 and TISTR 2075 were found to exhibit antimicrobial activity against *E. coli* O157:H7 DMST 12743 (100 AU.mL^{-1}), but no inhibitory effect against *S*. Typhimurium ATCC 13311 was detected. Based on these results, all adjusted CFS (pH 5.0 and 6.0) were found to lose their antimicrobial activity against the indicator strains. It is suggested that the antimicrobial activity of the test strains relies on acidity, lactic acid or other organic acids being produced (Tsai *et al.*, 2004; Lin *et al.*, 2006).

Viability of *L. plantarum* after exposure to simulated gastric and small intestinal juices

All 13 test strains were found to exhibit some tolerance ability to simulated gastric juice at pH 2.0 for 180 min (Table 4). Seven strains (TISTR 2073, TISTR 2074, TISTR 2075, TISTR 2076, TISTR 2077, TISTR 2078 and TISTR 2081) survived conditions of pH < 2.0 with a survival rate of 47.80–71.20%, while the other strains (TISTR 2070, TISTR 2071, TISTR 2072, TISTR 2079, TISTR 2080 and TISTR 2082) were sensitive to acid conditions and their viability was found to be completely destroyed after an exposure of 180 min. TISTR 2073 exhibited the highest tolerance with a viability loss of approximately 2.8 log CFU.mL^{-1}. Supporting the results reported by Pennacchia *et al*. (2004), *Lactobacillus* spp. recorded a survival rate of 60–80% in PBS pH 2.5 for 3 hr at 37 °C. However, according to Michida *et al*. (2006), *L. plantarum* NCIMB 8826 was found to have a

Table 2 Antibiotic susceptibility of the test strains of *L. plantarum*.

Antibiotic\ Test strain	Inhibition zone diameter (mm ± SD)												
	TISTR 2070	TISTR 2071	TISTR 2072	TISTR 2073	TISTR 2074	TISTR 2075	TISTR 2076	TISTR 2077	TISTR 2078	TISTR 2079	TISTR 2080	TISTR 2081	TISTR 2082
Chloramphenicol (30 µg)	23±1 (S)	25±0 (S)	24±0 (S)	28±1 (S)	25±1 (S)	22±2 (S)	22±1 (S)	22±1 (S)	27±2 (S)	23±2 (S)	24±2 (S)	24±1 (S)	24±1 (S)
Clindamycin (2 µg)	14±2 (R)	11±0 (R)	13±1 (R)	13±0 (R)	14±0 (R)	14±0 (R)	11±1 (R)	11±0 (R)	13±1 (R)	12±2 (R)	14±0 (R)	14±1 (R)	12±0 (R)
Rifampin (5 µg)	19±1 (I)	21±1 (S)	22±1 (S)	23±0 (S)	23±1 (S)	17±1 (I)	18±1 (I)	19±1 (I)	21±1 (S)	20±1 (I)	19±1 (I)	20±1 (I)	18±0 (I)
Kanamycin (30 µg)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)
Ciprofloxacin (5 µg)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)
Streptomycin (10 µg)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)
Penicillin (10 units)	21±1 (S)	20±1 (I)	18±0 (I)	21±1 (S)	20±0 (I)	16±0 (I)	20±1 (I)	21±0 (S)	20±0 (I)	21±2 (S)	20±1 (I)	18±0 (I)	21±1 (S)
Cepfoxitin (30 µg)	14±1 (R)	13±2 (R)	8±0 (R)	9±1 (R)	10±1 (R)	9±1 (R)	12±1 (R)	13±1 (R)	13±2 (R)	11±1 (R)	13±1 (R)	12±2 (R)	11±1 (R)
Oxacillin (1 µg)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)
Vancomycin (30 µg)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)	6±0 (R)

R = resistant; I = intermediate sensitive; S = sensitive.

Table 3 Antimicrobial activity of 13 strains of *L. plantarum* against *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311.

Strains	Antimicrobial activities* against indicator strains					
	E. coli O157:H7 DMST 12743			*S.* Typhimurium ATCC 13311		
	CFS**	CFS (pH 5.0)	CFS (pH 6.0)	CFS**	CFS (pH 5.0)	CFS (pH 6.0)
TISTR 2070	300	200	100	100	0	0
TISTR 2071	300	200	100	100	0	0
TISTR 2072	300	200	100	200	100	0
TISTR 2073	300	200	100	200	100	0
TISTR 2074	200	100	0	200	100	0
TISTR 2075	200	100	100	200	100	0
TISTR 2076	100	0	0	200	100	0
TISTR 2077	100	0	0	100	0	0
TISTR 2078	100	0	0	100	0	0
TISTR 2079	200	0	0	100	0	0
TISTR 2080	200	100	0	200	100	0
TISTR 2081	100	0	0	200	100	0
TISTR 2082	100	0	0	100	0	0

* = $AU.mL^{-1}$, where AU = arbitrary units; ** = non-neutralized cell-free supernatant.

Table 4 Viability of 13 strains of *L. plantarum* during exposure to simulated gastric juice pH 2.0 for 180 min.

Strain	Viable cell count (log $CFU.mL^{-1}$ ± SD)					Survival rate (% ± SD) after 180 min
	Initial	After exposure				
		30 min	60 min	90 min	180 min	
TISTR 2070	9.87±0.52	6.90±0.27	6.28±0.07	4.95±0.99	0.00±0.00	0.00±0.00^{e}
TISTR 2071	9.98±0.11	7.49±0.54	5.66±0.84	0.00±0.00	0.00±0.00	0.00±0.00^{e}
TISTR 2072	9.98±0.65	8.26±0.79	7.30±0.56	6.58±0.83	0.00±0.00	0.00±0.00^{e}
TISTR 2073	9.73±0.28	9.01±0.69	8.68±0.68	8.23±0.70	6.93±0.82	71.20±8.43^{a}
TISTR 2074	10.10±0.55	9.07±0.35	7.20±0.36	6.92±0.53	6.44±0.82	63.78±8.13^{abc}
TISTR 2075	9.75±0.36	9.08±0.94	8.18±0.93	7.32±0.67	4.66±0.12	47.80±1.20^{d}
TISTR 2076	9.97±0.52	9.23±0.26	7.75±0.75	7.65±0.58	5.39±0.84	54.05±8.40^{cd}
TISTR 2077	9.84±0.38	8.73±0.54	8.00±0.24	7.26±0.53	6.39±0.60	64.98±6.10^{ab}
TISTR 2078	10.03±0.40	8.44±0.83	6.42±0.13	5.84±0.07	5.47±0.18	54.56±1.82^{bcd}
TISTR 2079	9.59±0.52	4.42±0.51	3.59±0.63	0.00±0.00	0.00±0.00	0.00±0.00^{e}
TISTR 2080	9.85±0.54	4.37±0.07	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00^{e}
TISTR 2081	9.87±0.28	9.39±0.06	8.24±0.97	7.40±0.92	5.46±0.33	55.32±3.30^{bcd}
TISTR 2082	9.87±0.17	5.57±0.59	5.18±0.26	4.91±0.10	0.00±0.00	0.00±0.00^{e}

Values with different lower case letters (a–d) are significantly different by Duncan's multiple range test ($P < 0.05$).

higher loss of viability of approximately 8 log cycles after exposure to simulated gastric juice at pH 2.0 for 30 min.

The seven acid-tolerant strains of *L. plantarum* were selected to test their ability to survive in simulated small intestinal juice with 0.45% bile salt, as this was considered sufficient to determine any resistant strains (Buntin *et al*., 2008). As shown in Table 5, all strains selected were quite stable in simulated small intestinal juice with 0.45% bile salt for 240 min with viable cell reduction less than 27.00%. Three strains (TISTR 2073, TISTR 2077 and TISTR 2081) were observed to be the most bile tolerant with survival rates of 84.90, 89.96 and 89.31%, respectively. A similar finding was previously reported by Kacem *et al*. (2006) where *L. plantarum* OL9 and OL36 isolated from fermented olives showed the highest tolerance (65 and 59%, respectively). The viability of *L. plantarum* NCIMB 8826 isolated from human saliva was similarly found to decrease by 1.9 log CFU.mL^{-1} (Patel *et al*., 2004). According to Serrazanetti *et al.* (2009), the small intestinal juice tolerance of probiotic bacteria was strain dependent. Bile resistance of *Lactobacillus* spp. is related to the specific enzyme activity of bile salt hydrolase (BSH) which helps the hydrolysis of conjugated bile and thus reduces its toxic effects (du Toit *et al*., 1998).

Heat tolerance of *L. plantarum*

The heat tolerance of *L. plantarum* incubated at 65 °C for up to 60 min is shown in Table 6. Of the seven strains selected, TISTR 2075 exhibited the highest heat tolerance after heat exposure for 30 min with a survival rate of 98.51%

Table 5 Viability of seven selected strains of *L. plantarum* during exposure to simulated small intestinal juice pH 8.0 with 0.45% bile salt for 240 min.

Strain	Viable cells (log CFU.mL^{-1} ± SD)		Survival rate (% ± SD)
	Initial	After exposure	
TISTR 2073	9.66±0.09	8.19±0.02	84.90±0.02^{b}
TISTR 2074	9.78±0.04	7.14±0.05	73.14±0.36^{d}
TISTR 2075	9.95±0.05	7.72±0.08	77.51±0.90^{c}
TISTR 2076	9.37±0.02	7.30±0.08	78.00±0.10^{c}
TISTR 2077	9.63±0.17	8.72±0.02	89.96±0.98^{a}
TISTR 2078	9.56±0.21	7.29±0.15	76.79±0.73^{c}
TISTR 2081	9.72±0.09	8.65±0.10	89.31±0.55^{a}

Values with different lower case letters (a–d) are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 6 Viability of seven selected strains of *L. plantarum* after exposure to 65 °C for 60 min.

Strain	Viable cells (log CFU.mL^{-1} ± SD)			Survival rate (% ± SD) after 30 min
	Initial	After exposure		
		30 min	60 min	
TISTR 2073	9.43±0.09	6.17±0.18	0.00±0.00	65.41±1.95^{d}
TISTR 2074	9.48±0.11	4.82±0.05	0.00±0.00	50.83±0.49^{e}
TISTR 2075	9.42±0.06	9.28±0.03	0.00±0.00	98.51±0.34^{a}
TISTR 2076	9.45±0.12	7.67±0.13	0.00±0.00	81.21±1.37^{c}
TISTR 2077	9.44±0.13	8.74±0.16	0.00±0.00	92.55±1.71^{b}
TISTR 2078	9.51±0.09	7.58±0.07	0.00±0.00	79.75±0.74^{c}
TISTR 2081	9.51±0.10	1.59±0.22	0.00±0.00	16.71±2.36^{f}

Values with different lower case letters (a–f) are significantly different by Duncan's multiple range test ($P < 0.05$).

followed by TISTR 2077 (92.55%), TISTR 2076 (81.21%) and TISTR 2078 (79.75%). In contrast, TISTR 2081 was found to be very sensitive to heat with a survival rate of 16.71%. However, no strains remained viable after 60 min of incubation. Compared to Ding and Shah (2007), a higher loss of viability was observed in the encapsulated and free cells of *L. plantarum* after heat treatment at 65 °C for 30 min, with approximately 2 and 4 log CFU.mL^{-1}, respectively. In addition, Kim *et al.* (2001) suggested that a temperature at 60 °C was considered as the lethal temperature because the viability of *L. acidophilus* was significantly reduced but not all cells were killed. Therefore, it could be claimed that TISTR 2075, TISTR 2076, TISTR 2077 and TISTR 2078 are thermotolerant strains.

CONCLUSION

L. plantarum TISTR 2075 isolated from fermented vegetables was found to meet all the criteria outlined above and could be considered as probiotic . This strain showed strong autoaggregation and cell surface hydrophobicity which are related to the adhesion ability to intestinal cells and it also had positive coaggregation with *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311 linked to the ability to interact closely with pathogens. In addition, the strain was resistant to some antibiotics tested which belonged to the major classes of antibiotics used in human clinical therapy. Furthermore, it had antimicrobial activity against both pathogens and could survive under gastrointestinal tract conditions. Additionally, it was able to withstand a high temperature of 65 °C for 30 min which is a desirable characteristic for industrial strains as it could have a better chance of remaining viable during the drying process required for prolonged storage. Therefore, *L. plantarum* TISTR 2075 may be regarded as a potential probiotic candidate. Clinical trials on the potential health benefits to consumers should be further investigated.

ACKNOWLEDGEMENT

This research was financially supported by the Office of the Higher Education Commission, the Center of Advanced Studies for Agriculture and Food (CASAF) and the Kasetsart University Institute for Advanced Studies (KUIAS).

LITERATURE CITED

Alemu, M., D. Kraykaw, S. Ohmomo and S. Nitisinprasert. 2002. Screening and identification of thermophilic lactic acid bacteria producing antibacterial substances determined by OMP analysis. **Kasetsart J. (Nat. Sci.)** 37: 493–505.

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

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck. 1966. Antibiotic susceptibility testing by a standard single disc method. **Am. J. Clin. Pathol.** 45: 493–496.

Brinques, G.B. and M.A.Z. Ayub. 2011. Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt. **J. Food Eng.** 103(2): 123–128.

Buntin, N., S. Chanthachum and T. Hongpattarakere. 2008. Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics **Songklanakarin J. Sci. Technol.** 30: 141–148.

Candela, M., F. Perna, P. Carnevali, B. Vitali, R. Ciati, P. Gionchetti, F. Rizzello, M. Campieri and P. Brigidi. 2008. Interaction of probiotic *Lactobacillus* and *Bifidobacterium* strains with human intestinal epithelial cells:

Adhesion properties, competition against enteropathogens and modulation of Il-8 production. **Int. J. Food Microbiol.** 125(3): 286–292.

Cebeci, A. and C. Gürakan. 2003. Properties of potential probiotic *Lactobacillus plantarum* strains. **Food Microbiol.** 20 (5): 511–518.

de Vries, M.C., E.E. Vaughan, M. Kleerebezem and W.M. de Vos. 2006. *Lactobacillus plantarum*-survival, functional and potential probiotic properties in the human intestinal tract. **Int. Dairy J.** 16: 1018–1028.

Del Re, B., B. Sgorbati, M. Miglioli and D. Palenzona. 2000. Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. **Lett. Appl. Microbiol.** 31: 438–442.

Ding,W.K. and N.P. Shah. 2007. Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. **J. Food Sci.** 72(9): M446–M450.

du Toit, M., C.M.A.P. Franz, L.M.T. Dicks, U. Schillinger, P. Haberer, B. Warlies, F. Ahrens and W.H. Holzapfel. 1998. Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. **Int. J. Food Microbiol.** 40: 93–104.

FAO/WHO. 2002. **Guidelines for the Evaluation of Probiotics in Food.** Working group report. Food and Agricultural Organization of the United Nations and the World Health Organization. London, Ontario, Canada.

Garriga, M., M. Pascual, J.M. Monfort and M. Hugas. 1998. Selection of *Lactobacilli* for chicken probiotic adjuncts. **J. Appl. Microbiol.** 84(1): 125–132.

Giraffa, G., N. Chanishvili and Y. Widyastuti. 2010. Importance of lactobacilli in food and feed biotechnology. **Res. Microbiol.** 161(6): 480–487.

Handley, P.S., D.W.S. Harty, J.E. Wyatt, C.R. Brown, J.P. Doran and A.C.C. Gibbs. 1987. A comparison of the adhesion, coaggregation and cell-surface hydrophobicity properties of fibrillar and fimbriate strains of *Streptococcus salivarius*. **J. Gen. Microbiol.** 133(11): 3207–3217.

Herreros, M.A., H. Sandoval, L. González, J.M. Castro, J.M. Fresno and M.E. Tornadijo. 2005. Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). **Food Microbiol.** 22(5): 455–459.

Huang, Y. and M.C. Adams. 2004. *In vitro* assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. **Int. J. Food Microbiol.** 91: 253–260.

Kacem, M., H. Zadi-Karam and N. Karam. 2006. Detection and characterization of a novel bacteriocin produced by *Lactobacillus plantarum* OL9 against *Erwinia chrysanthemi* strains Mu'tah Lil-Buhuth wad-Dirasat. **Nat. Appl. Sci. Ser. (Jordan)** 21: 159–171.

Kailasapathy, K. and J. Chin. 2000. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. **Immunol. Cell Biol.** 78(1): 80–88.

Kaushik, J.K., A. Kumar, R.K. Duary, A.K. Mohanty, S. Grover and V.K. Batish. 2009. Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum.* **PLoS ONE** 4(12): e8099.

Kim, W.S., L. Perl, J.H. Park, J.E. Tandianus and N.W. Dunn. 2001. Assessment of stress response of the probiotic *Lactobacillus acidophilus*. **Curr. Microbiol.** 43: 346–350.

Kos, B., J. Šušković, S. Vuković, M. Šimpraga, J. Frece and S. Matošić. 2003. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. **J. Appl. Microbiol.** 94(6): 981–987.

Kosin, B. and S.K. Rakshit. 2006. Microbial

and processing criteria for production of probiotics:A review. **Food Technol. Biotechnol.** 44(3): 371–379.

Lin, W.-H., C.-F. Hwang, L.-W. Chen and H.-Y. Tsen. 2006. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. **Food Microbiol.** 23: 74–81.

Mathur, S. and R. Singh. 2005. Antibiotic resistance in food lactic acid bacteria - a review. **Int. J. Food Microbiol.** 105(3): 281–295.

Michida, H., S. Tamalampudi, S.S. Pandiella, C. Webb, H. Fukuda and A. Kondo. 2006. Effect of cereal extracts and cereal fiber on viability of *Lactobacillus plantarum* under gastrointestinal tract conditions. **Biochem. Eng. J.** 28: 73–78.

Patel, H. M., S.S. Pandiella, R.H. Wang and C. Webb. 2004. Influence of malt, wheat, and barley extracts on the bile tolerance of selected strains of lactobacilli. **Food Microbiol.** 21: 83–89.

Pennacchia, C., D. Ercolini, G. Blaiotta, O. Pepe, G. Mauriello and F. Villani. 2004. Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. **Meat Sci.** 67: 309–317.

Prado, F.C., J.L. Parada, A. Pandey and C.R. Soccol. 2008. Trends in non-dairy probiotic beverages. **Food Res. Int.** 41(2): 111–123.

Serrazanetti, D.I., M.E. Guerzoni, A. Corsetti and R. Vogel. 2009. Metabolic impact and potential exploitation of the stress reactions in lactobacilli. **Food Microbiol.** 26(7): 700–711.

Sirilun, S., C. Chaiyasut, D. Kantachote and P. Luxananil. 2010. Characterisation of non human origin probiotic *Lactobacillus plantarum* with cholesterol-lowering property. **Afr. J. Microbiol. Res.** 4(10): 994–1000.

Taheri, H.R., F. Tabandeh, H. Moravej, M. Zaghari, M. Shivazad and P. Shariati. 2009a. Potential probiotic of *Lactobacillus johnsonii* LT171 for chicken nutrition. **Afr. J. Biotechnol.** 8(21): 5833–5837.

Taheri, H.R., H. Moravej, F.Tabandeh, M. Zaghari and M. Shivazad. 2009b. Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. **Poult. Sci.** 88: 1586–1593.

Tsai, C.C., L.F. Huang, C.C. Lin and H.Y. Tsen. 2004. Antagonistic activity against *Helicobacter pylori* infection in vitro by a strain of *Enterococcus faecium* TM39. **Int. J. Food Microbiol.** 96: 1–12.

Vlková, E., V. Rada, P. Popelárová, I. Trojanová and J. Killer. 2006. Antimicrobial susceptibility of *Bifidobacteria* isolated from gastrointestinal tract of calves. **Livest. Sci.** 105(1–3): 253–259.

Zhou, J.S., C.J. Pillidge, P.K. Gopal and H.S. Gill. 2005. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. **Int. J. Food Microbiol.** 98(2): 211–217.