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Full Length Research Paper

Screening of *Lactobacillus plantarum* isolated from fermented idli batter for probiotic properties

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Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. The objective of this study was to screen eight potential probiotic *Lactobacillus plantarum* strains from fermented idli batter using *in vitro* assays such as bile tolerance, acid tolerance, transit tolerance in the upper human gastrointestinal tract, auto-aggregation, co-aggregation, hydrophobicity, susceptibility to various antibiotics, bile salt hydrolase assay, cholesterol assimilation and hemolysis. The isolates were able to tolerate up to 0.3% of bile for 4 to 6 h and pH 2.5, 3.5, 4.5, 6.5, 7.5 and 8.5. The isolates were able to resist growth against gastric and intestinal fluid. The auto-aggregation of the different *L. plantarum* strains ranged from 65 to 80% in all the isolates. The co-aggregation with pathogens like *Listeria monocytogenes* (MTCC 657) and *Escherichia coli* (MTCC 728) ranged from 51 to 64%, however, low levels of co-aggregation were observed in *L. plantarum* (MTCC 6161) and *L. rhamnosus* (MTCC 1408) ranging from 32 to 46% and hydrophobicity from 49 to 77%. The isolates showed resistance towards antibiotics like gentamycin, ciprofloxacin, nalidixic acid and norfloxacin. All the isolates showed bile salt hydrolase activity with cholesterol lowering capacity, the highest being 73% by *L. plantarum* JJ 18. The isolates possessed β-galactosidase activity exhibiting 322 to 1000 MU of enzyme activity. No isolates showed hemolysis activity. Thus, the different *L. plantarum* isolates exhibited probiotic potential which would attribute beneficial effect to mankind.

Key words: *Lactobacillus plantarum*, probiotics, cholesterol, β-galactosidase.

INTRODUCTION

The increase in bacterial resistance to various antibiotics has stimulated investigations around the world to improve disease control strategies which led to the discovery of new vaccines and non-specific immune-stimulants (Balcazar et al., 2008). Thus, there is a growing interest in the use of probiotic bacteria worldwide for their various beneficial influence on animal and human health (Guarner and Malagelada, 2003; Mercenier et al., 2003; Lee et al., 2008), Lactic acid bacteria (LAB) are Generally Recognized as Safe (GRAS) organisms. LAB are characterised by their production of lactic acid and are predominant participants in many industries and furthermore, LAB are indigenous inhabitants of the human gastro intestinal tract (GIT), and are thought to be dominant in the small intestine (Marco et al., 2006). The genus Lactobacillus is the largest group among the Lactobacteriaceae, and contains over 100 species (Canchaya et al., 2006). The best-studied probiotic strains among the Lactobacteriaceae, involves the species *Lactobacillus acidophilus*, *L. fermentum*, *L. plantarum*, *L. brevis*, *L. jensenii*, *L. casei*, *L. delbrueckii*, *L. vaginalis* and *L. salivarius* (De Vries et al., 2006; Galdeano et al., 2007; Ranadheera et al., 2010).

Probiotics are defined as 'live microorganisms that when administered in adequate amounts, confer a health benefit on the host (Reid et al., 2003). The principle requisite for selection of a good probiotic includes product

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safety for human and animal consumption (GRAS) and survival in the gastrointestinal tract (GIT) (Hyronimus et al., 2000). The probiotic strains must possess the ability to overcome the extremely low pH and the detergent effect of bile salts, and arrive at the site of action in a viable physiological state (Chou and Weimer, 1999). They should be capable of co-aggregation, resistant to gastro intestinal fluid and adhere to the intestinal mucosa (Jacobsen et al., 1999; Dunne et al., 2001). However, besides the various essential characteristics, the organisms should exhibit health benefits with functional properties. Various functional characteristics have been developed by the organisms. Clinically proven, various health effects have been reported for lactobacilli, such as cholesterol reduction, diarrhoea prevention, enhancement of lactose intolerance symptoms, anticancer effects, synthesis and enhancing the bioavailability of nutrients and immune-modulatory effects, all of which are considered functional aspects of probiotic criteria. In order to exert their beneficial effect, probiotics must survive in the gastrointestinal (GI) tract, persist in the host, and provide safety for the consumer (De-Vries et al., 2006).

This present study was designed to screen the various *L. plantarum* strains isolated from fermented idli batter (Accession nos. JN573601 to JN573608) for probiotic properties which may exert beneficial effects for mankind.

MATERIALS AND METHODS

Isolates

The following *L. plantarum* strains isolated from fermented idli batter were used for this study: *L. plantarum* JJ 18 (JN573601), *L. plantarum* subsp. *plantarum* JJ 60 (JN573602), *L. plantarum* JJ 55 (JN573603), *L. pentosus* JJ 58 (JN573604), *L. plantarum* JJ 29 (JN573605), *L. plantarum* JJ 30 (JN573606), *L. plantarum* subsp. *argentoratensis* JJ 24 (JN573607) and *L. plantarum* JJ 22 (JN573608).

Bile tolerance

Strains were grown in De Man, Rogosa & Sharpe (MRS) broth containing 0.05, 0.1, 0.3, 0.6, and 1% of bile. The assay was conducted in sterile flat-bottom 96-well microtitre plates. Each well was filled with 180 µl of the medium and inoculated with 20 µl of the cultures obtained in MRS broth (OD_{600nm} = 0.2) at 37°C. Optical density readings were recorded at 600 nm every hour for 12 h, while cultures grown in MRS broth containing 0% bile served as the control (Todorov et al., 2011).

pH tolerance

Strains were grown in MRS broth adjusted to pH 2, 2.5, 3.5, 7.5 and 8.5. The assay was conducted in sterile flat-bottom 96-well microtitre plates. Each well was filled with 180 µl of the medium and inoculated with 20 µl of the cultures obtained in MRS broth (OD_{600nm} = 0.2) at 37°C. Optical density readings were recorded at 600 nm every hour for 12 h, while cultures grown in MRS broth pH 6.5 served as the control (Todorov et al., 2011).

Resistance to gastric acidity and bile salts

The resistance to artificial gastric and intestinal fluids were investigated for the strains. Pure cultures (10^8 CFU ml^{-1}) were exposed to artificial gastric fluid (NaCl, 0.72 g l^{-1}; KCl, 0.05 g l^{-1}; $NaHCO_3$, 0.37 g l^{-1}; pepsin, 0.3 g l^{-1}) adjusted to pH 3.0 with HCl 1 M and to pH 7.0 with NaOH 1 M as the control condition for 0, 90 and 180 min. After 180 min of incubation in artificial gastric fluid at pH 3.0 and in control condition (pH 7.0), the bacteria were exposed to artificial intestinal fluid (0.1% w/v pancreatin and 0.3% w/v Oxgall bile salts, pH 8.0) for 0, 90 and 180 min. Total viable counts were determined on MRS agar after a serial 10-fold dilution in PBS (Ripamonti et al., 2011).

Auto-aggregation

Strains were grown in MRS broth for 24 h at 37°C. The cells were harvested, washed and resuspended in sterile PBS and adjusted to OD of 1 at 600 nm. After 60 min, the cultures were centrifuged at 300 g for 2 min at 20°C and the OD_{600nm} was recorded. Auto-aggregation was determined using the following equation.

$$\%\,\text{Auto-aggregation} = \left[\frac{(OD_0 - OD_{60})}{OD_0}\right] \times 100$$

OD_0 refers to the initial OD, and OD_{60} refers to the OD determined after 60 min (Todorov et al., 2011).

Co-aggregation

To evaluate co-aggregation, strains *L. plantarum* (MTCC 6160) and *L. rhamnosus* (MTCC 1408) grown in 10 ml of MRS broth, *Listeria monocytogenes* (MTCC 657) and *E. coli* (MTCC 728) grown in Tryptic soya broth (sensitive to the bacteriocins) at 37°C were used. Cells were harvested after 24 h, washed, resuspended in sterile PBS. One millilitre of each cell suspension was transferred and the OD_{600nm} recorded over 60 min using a spectrophotometer. Cells were harvested at 300 g for 2 min at 20°C and the OD_{600nm} of the supernatant was determined. Co-aggregation was calculated using the following equation.

$$\%\,\text{Co-aggregation} = \left[\frac{(OD_{tot} - OD_s)}{OD_{tot}}\right] \times 100$$

OD_{tot} refers to the initial OD taken immediately after the relevant strains were paired. OD_S refers to the OD of the supernatant after 60 min (Todorov et al., 2011).

Hydrophobicity

The test for bacterial adhesion to hydrocarbons (BATH) was carried out. Strains were grown in MRS broth at 37°C for 18 h. Cells were harvested and washed twice with PBS and resuspended in the same solution and the optical density (OD_{600nm}) was determined. A sample of 1.5 ml cell suspension was added to 1.5 ml of n-hexadecane and vortexed for 2 min. The aqueous and organic phases were allowed to separate for 30 min at room temperature. One millilitre of the aqueous phase was removed and the optical density (OD_{600nm}) was determined. The experiment was repeated and the average optical density value determined. The percentage hydrophobicity was calculated as follows;

$$\%\ \text{Hydrophobicity} = \left[\frac{\left(OD_{600\ \text{reading}\,1} - OD_{600\ \text{reading}\,2}\right)}{OD_{600\ \text{reading}\,1}}\right] \times 100$$

The experiments were conducted in triplicates (Lee et al., 2011).

Antibiotic susceptibility test

The antibitotic susceptibility was determined semi quantitatively by using disc diffusion method. Various classes of antibiotics were chosen for the study. Susceptibility to inhibitors of cell wall synthesis with penicillins and cephalosporins were checked. The susceptibility against inhibitors of protein synthesis was also checked with tetracyclines and aminoglycosides. The susceptibility towards various inhibitors of nucleic acid synthesis, cytoplasmic membrane functions and urinary tract antiseptics were also checked (Charteris et al., 1998).

Bile salt hydrolase

Strains were screened for bile salt hydrolase (BSH) activity by streaking culture grown in MRS broth unto BSH screening medium which consisted of MRS agar supplemented with 0.5% (w/v) sodium salt of TDCA (taurodeoxycholic acid) and 0.37 g $CaCl_2$/l. Plates were incubated anaerobically in an anaerobic jar at 37°C. The BSH activity was semi-quantified by the precipitation zones (Lee et al., 2011).

Screening for cholesterol-lowering capacity

Freshly prepared MRS broth supplemented with 0.3% (w/v) bile salt was used for the assay. Three milliliter (3 ml) of 95% ethanol and 2 ml of 50% potassium hydroxide were added to 1 ml of supernatant of the samples. The contents of the tubes were mixed after the addition of each component and then heated for 10 min in a 60°C water bath. After cooling, 5 ml of hexane was added into each tube and mixed thoroughly. One milliliter (1 ml) aliquot of distilled water was added, mixed and tubes were allowed to stand for 10 min at room temperature to permit phase separation. A 3 ml aliquot of hexane layer was transferred to a clean tube and the hexane evaporated under the flow of nitrogen gas. A 4 ml sample of freshly prepared o-phthalaldehyde in acetic acid (0.5 mg ml^{-1}) was added to each tube and they were allowed to stand at room temperature for 10 min. Following the addition of 2 ml concentrated sulphuric acid and standing for additional 10 min, the absorbance at 550 nm was read against reagent blank. Absorbance values were compared (Mathara et al., 2008).

β-Galactosidase activity

Overnight cultures of the strains were harvested and washed in 60 mM Na_2HPO_4/40 mM NaH_2PO_4 buffer (pH 7.0) and inoculated (1% v/v) in MRS-lac broth. Cultures were incubated at 37°C for 24 h. Cells were harvested and washed twice as previously described and A_{560} nm was adjusted to approximately 1.0 with the same buffer. One milliliter of the cell suspension was permeabilized with 50 ml of toluene : acetone (1:9 v/v) solution, vortexed for 7 min and immediately assayed for β-galactosidase activity. An aliquot of 100 µl of the permeabilized cell suspension was taken and 900 µl of phosphate buffer and 200 µl of o-nitrophenyl-β-D-galactopyranoside (ONPG) (4 mg ml^{-1}) were added to the cell suspension. Tubes were placed into a water bath at 37°C for 15 min. Finally, 0.5 ml of 1 M Na_2CO_3 was added to stop the reaction. Absorbance values at both 420 and 560 nm were recorded for each tube. β-Galactosidase activity was calculated in Miller units as follows:

$$\beta\text{-galactosidase activity} = 1000 \times \left[\frac{\left(A_{420} - 1.75 \times A2_{560}\right)}{\left(15\text{min} \times 1\text{ml} \times A1_{560}\right)}\right]$$

Where $A1_{560}$ is the absorbance just before assay and $A2_{560}$ is the absorbance value of the reaction mixture (Vinderola and Reinheimer, 2003)

Hemolysis

The isolates were streaked on MRS agar supplemented with 5% blood to check for hemolysis (Mourad and Eddine, 2006).

RESULTS AND DISCUSSION

All the isolates showed growth in the absence of bile as well as in the presence of 0.05, 0.1, and 0.3% of bile, whereas no growth was observed in higher percentage of 0.6 and 1 as illustrated in Figure 1. The physiological concentration of human bile ranges from 0.3 to 0.5% (Dunne et al., 1999; Zavaglia et al., 1998). Various lactobacilli were resistant to bile (Charteris et al., 1998a). In our present study, the *L. plantarum* strains were able to resist the physiological bile salt concentration. It has been reported earlier that *L. plantarum* strains were able to tolerate physiological bile indicating probiotic potential (Cebeci and Gurakan, 2003). However, isolates in the present study were not able to tolerate higher percentage of 0.6 and 1% as reported earlier by Todorov et al. (2008). Good growth of all tested *L. plantarum* strains was recorded in MRS broth with pH values of 3.5, 4.5, 7.5 and 8.5. However, the isolates were less tolerant to pH 2, 2.5 as illustrated in Figure 2. About 2.5 l of gastric juice at a pH of approximately 2.0 is secreted each day in the stomach, and hence it is a requisite for the isolates to tolerate acidic pH (Charteris et al., 1998a). The acidic pH causes destruction of most microorganisms ingested. In this sense, resistance to human gastric transit is an important selection criterion for probiotic microorganisms (Charteris et al., 1998b).

The effects of gastric and intestinal digestion on the survival of isolated strains were studied. No significant effect was observed as the viability was similar in pH 3 and 7, indicating that the *L. plantarum* strains showed great resistance on exposure to gastric fluid at pH 3.0 and 7.0 as shown in Table 1. The gastric transit was monitored from 0 to 90 min and then to 180 min. The reason for 90 min of incubation time in acidic broth is that the time from entrance to release from the stomach is 90 min. However, further digestive processes increase passage time (Chou and Weimer, 1999). A significant decrease in the viability was observed in intestinal juice between 270 to 360 min. Thus, all the isolates were able to survive conditions mimicking the gastro intestinal

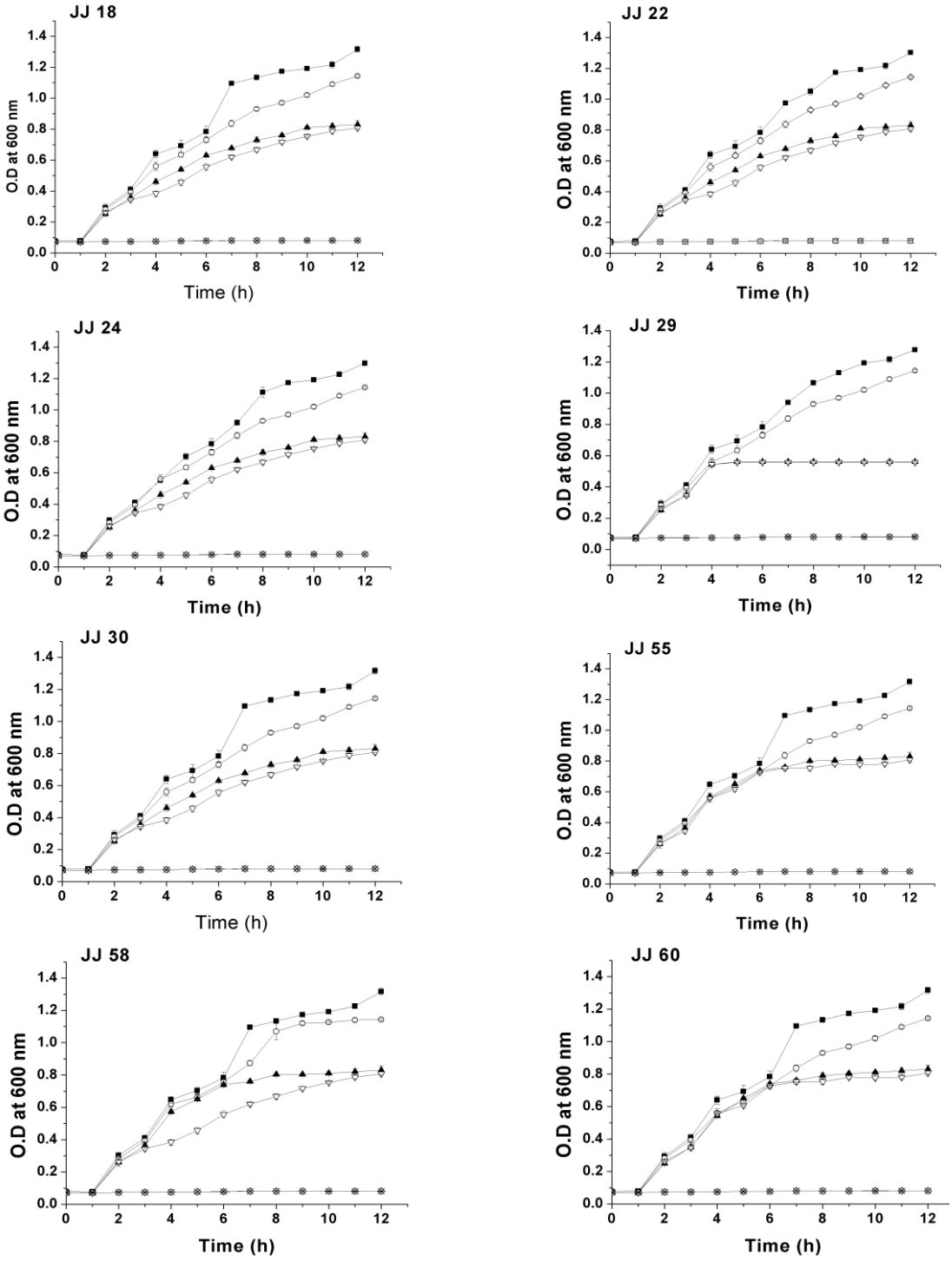

Figure 1. Bile tolerance of the isolates. The experiment was performed in triplicates; ■ 0% ○ 0.05% ▲ 0.1% ▽ 0.3% ◇ 0.6% × 1% .

environment. *L. plantarum* has a proven ability to survive gastric transit and colonize the gut, with an apparent safety to the consumer (De-Vries et al., 2006).

Bacterial aggregation between microorganisms of the same strain (auto-aggregation) or between genetically different strains (co-aggregation) is of considerable importance in several ecological niches, especially in the human gut. In the present study auto-aggregation of the

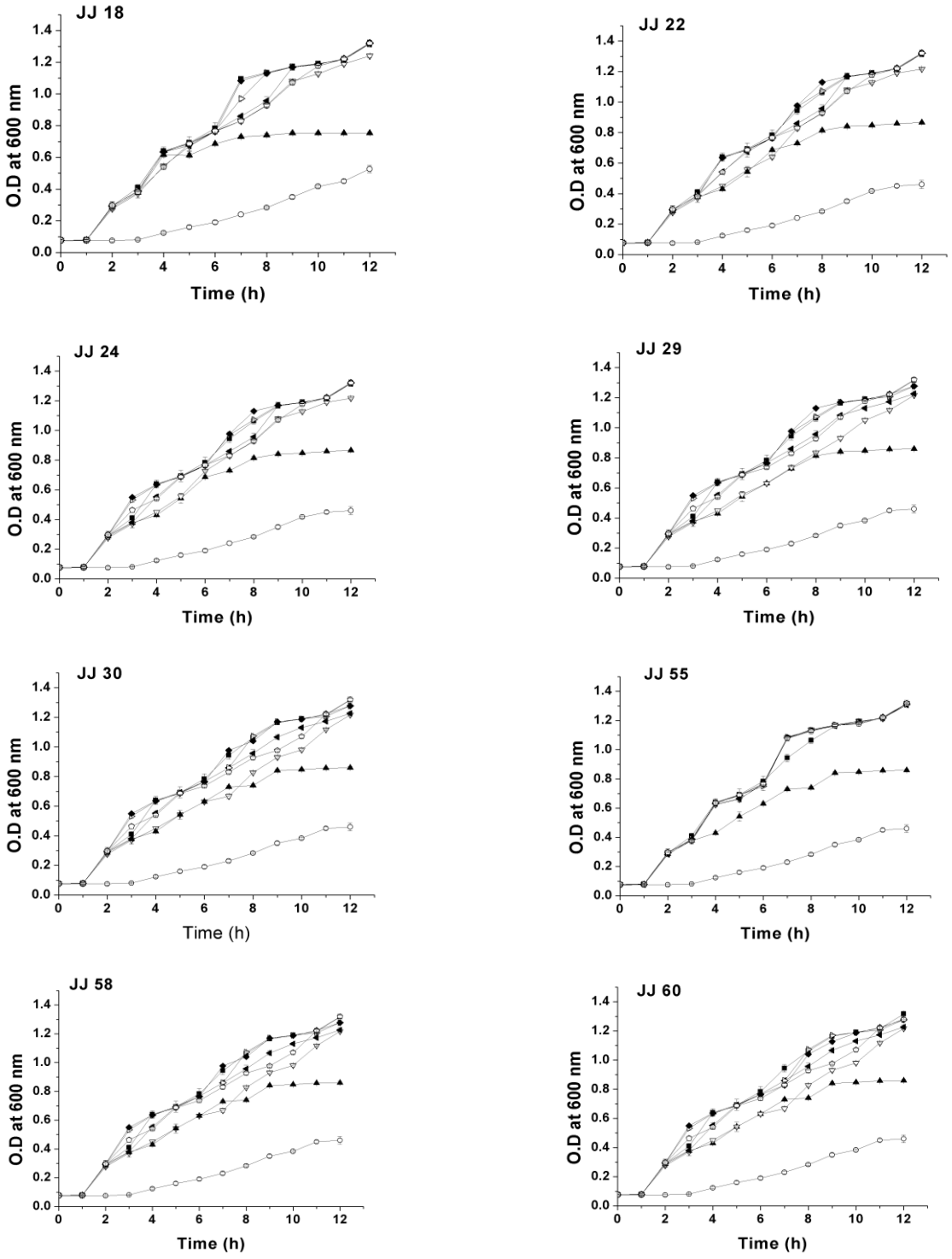

Figure 2. pH tolerance of the isolates. The experiment was performed in triplicates;

different *L. plantarum* strains ranged from 65 to 80% in all the isolates as shown in Table 2. The co-aggregation with pathogens like *Listeria monocytogenes* (MTCC 657) and *E. coli* (MTCC 728) ranged from 51 to 64% as shown in Table 2. However, low levels of co-aggregation were observed in *L. plantarum* (MTCC 6161) and *L. rhamnosus*

Table 1. Effect of artificial gastric fluid at pH 3 and 7 followed by introduction of artificial intestinal fluid at 180 min on the survival of the isolates.

Isolate No.	Log CFU ml^{-1}*				
	Gastric fluid			Intestinal fluid	
	0 min	90 min	180 min	270 min	360 min
JJ 18 pH 7	8.15±0.4	8.19±0.1	8.25±0.2	7.53±0.3	7.25±0.1
pH 3	8.05±0.2	8.11±0.1	8.16±0.1	7.24±0.1	7.15±0.1
JJ 22 pH 7	8.13±0.5	8.19±0.1	8.26±0.3	7.47±0.1	7.28±0.1
pH 3	8.07±0.1	8.11±0.1	8.16±0.1	7.25±0.1	7.14±0.1
JJ 24 pH 7	8.12±0.2	8.20±0.2	8.26±0.2	7.54±0.1	7.31±0.1
pH 3	8.09±0.1	8.13±0.1	8.15±0.1	7.27±0.1	7.13±0.1
JJ 29 pH 7	8.13±0.4	8.18±0.1	8.24±0.2	7.44±0.1	7.24±0.1
pH 3	8.10±0.2	8.13±0.5	7.91±0.1	6.96±0.7	6.72±0.1
JJ 30 pH 7	8.14±0.1	8.15±0.2	8.24±0.1	7.53±0.1	7.26±0.2
pH 3	8.06±0.2	8.13±0.1	7.83±0.5	6.82±0.2	6.64±0.5
JJ 55 pH 7	8.15±0.5	8.16±0.1	8.26±0.1	7.45±0.3	7.24±0.1
pH 3	8.07±0.1	8.12±0.2	8.14±0.1	7.24±0.1	7.13±0.5
JJ 58 pH 7	8.17±0.2	8.19±0.3	8.24±0.1	7.55±0.2	7.31±0.1
pH 3	8.09±0.1	8.11±0.1	8.15±0.5	7.24±0.1	7.18±0.1
JJ 60 pH 7	8.17±0.3	8.19±0.1	8.26±0.1	7.48±0.3	7.24±0.1
pH 3	8.06±0.2	8.13±0.5	8.19±0.1	7.25±0.1	7.15±0.1

*The values are Mean ± SD of three independent experiments performed in duplicates.

Table 2. Auto- aggregation, co-aggregation and hydrophobicity of the different isolates.

Isolate No.	Auto-aggregation (%)	Co-aggregation (%)				Hydrophobicity (%)
		Lactobacillus rhamnosus (MTCC 1408)	*Lactobacillus plantarum* (MTCC 6161)	*Listeria monocytogenes* (MTCC 657)	*Escherichia coli* (MTCC 728)	
JJ 18	66±0.3	43±0.3	40±0.6	63±0.2	57±0.3	49±0.2
JJ 22	68±0.5	41±0.2	40±0.4	53±0.9	51±0.4	56±0.3
JJ 24	65±0.3	40±0.3	35±0.3	58±0.2	58±0.4	49±0.4
JJ 29	80±0.5	39±0.5	38±0.3	54±0.4	63±0.3	77±0.3
JJ 30	69±0.3	44±0.3	41±0.3	58±0.4	58±0.3	49±0.3
JJ 55	67±0.4	46±0.3	37±0.3	59±0.3	61±0.3	52±0.4
JJ 58	77±0.4	41±0.2	32±0.2	64±0.3	63±0.3	72±0.2
JJ 60	72±0.4	43±0.4	39±0.3	62±0.5	58±0.4	53±0.5

The values are Mean ± SD of three independent experiments performed in duplicates.

(MTCC 1408) with 32 to 46% as shown in Table 2. Aggregation is an important feature for biofilm formation. However, co-aggregation between LAB and other cells, especially *L. monocytogenes*, may be considered a positive characteristic, as it is one of the steps required for the elimination of non desirable strains from the GIT (Todorov and Dicks, 2008). Auto-aggregation and co-aggregation are strain-specific and most probably involve species-specific surface proteins. *L. plantarum* has a number of genes encoding for surface proteins that could function in recognition of, or binding to components in the environment. Several of these genes are homologous to proteins with predicted functions, such as mucus binding, aggregation promoting and intracellular adhesion (Kleerebezem et al., 2003).

Adhesion is a complex process involving non-specific (hydrophobicity) and specific ligand-receptor mechanisms. The *L. plantarum* strains in our present study showed hydrophobicity from 49 to 77% with hexadecane as shown in Table 2. The determination of microbial adhesion to hexadecane as a way to estimate the ability of a strain to adhere to epithelial cells is a valid qualitative phenomenological approach (Kiely and Olson, 2000). Adherence of bacterial cells is usually related to cell surface characteristics. Cell surface hydrophobicity is a nonspecific interaction between microbial cells and host. The initial interaction may be weak, often reversible and precedes subsequent adhesion processes mediated by more specific mechanisms involving cell surface proteins and lipoteichoic acids (Rojas et al., 2002; Ross and Jonsson, 2002). Bacterial cells with a high hydrophobicity usually present strong interactions with mucosal cells. The hydrophobicity values of 75 to 80% for *L. plantarum* ST664BZ, which were higher than those for *L. rhamnosus* GG (55%), were recorded by Todorov et al. (2008). The *L. plantarum* in the present study exhibited resistance towards inhibitors of protein synthesis (gentamycin), inhibitors of nucleic acid synthesis (ciprofloxacin, nalidixic acid, norfloxacin), and inhibitor of cytoplasmic membrane functions (colistin) as depicted in Table 3. *Lactobacillus* have natural resistance to the above antibiotics (Mathur and Singh, 1995). The strains were sensitive to various antibiotics. Resistance may be inherent to a bacterial genus or species, but may also be acquired through exchange of genetic material, mutations and the incorporation of new genes (Ammor et al., 2007). Potential probiotic LAB may act as reservoir of antibiotic resistance genes, and horizontal gene transfer to the other bacteria present in the human GIT is possible. Special purpose probiotics for use in combination with antibiotics have been developed through the introduction of multiple resistances to the bacteria (Mathur and Singh, 2005).

Bile salt deconjugation is an important characteristic, as it could play a role in maintaining the equilibrium of the gut microflora in reducing serum cholesterol (Corzo and Gilliland, 1999) and in the production of a detergent shock protein that enables lactobacilli to survive exposure to bile. The high bile salt hydrolase activity of lactobacilli might have some role in the reduction of the serum cholesterol level. Bile excretion is a major route of eliminating cholesterol from the body, as well as one of the important pathways of cholesterol metabolism (Liong and Shah, 2005). Most conjugated bile salts excreted (about 97%) are reabsorbed from the small intestine and returned to the liver through the hepatic portal circulation. All the *L. plantarum* strains in this study showed good BSH activity, thereby suggesting ability for cholesterol reduction (Table 4). Experiments were performed to determine cholesterol lowering effect. The lactobacilli strains were able to reduce 41 to 73% of cholesterol (Table 4). Among the eight lactobacilli, JJ18 showed highest cholesterol reducing activity. The possible mechanisms underlying the ability of *L. plantarum* strains in this study to remove cholesterol from the media would be co-precipitation of cholesterol with free bile salts derived from deconjugation of bile salts as reported by Klaver and van der Meer (1993). As the solubility of cholesterol decreases due to deconjugation of bile salts by the *L. plantarum*, strains will likely be excreted through faeces.

Lactose intolerance has been recognized for many years as a common problem in many children and most adults throughout the world (Heyman, 2000). The *L. plantarum* strains showed β-galactosidase activity. The isolates were able to show activity of 812 to 1000 MU, the highest activity shown by *L. pentosus* JJ 58 except for *L. plantarum* JJ 29 and JJ 30 which showed 518 and 322 MU, respectively (Table 4). Therefore, by addition of lactobacilli producing β-galactosidase as probiotic to food products could help to alleviate lactose intolerance symptoms. Thus, strains producing β-gal has gained importance for potential applications as probiotic cultures in industry or as producers of the prebiotic ingredients galacto-oligosaccharides (Ibrahim and O'Sullivan, 2000). Finally, no isolates showed haemolytic activity, indicating absence of hemolysin activity (Table 4).

Conclusion

The different *L. plantarum* strains screened in this study possessed probiotic properties. The strain *L. plantarum* JJ18 showed a higher cholesterol removal capacity from media, and tolerance towards acid and bile, indicating that it may be able to serve as a probiotic strain after further characterisation is completed by means of animal models and clinical tests.

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Table 3. Antibiotic susceptibility of the different isolates.

Antibiotics	JJ 18	JJ 22	JJ 24	JJ 29	JJ 30	JJ 55	JJ 58	JJ 60
Inhibitors of cell wall synthesis								
Penicillins								
Penicillin G (10 μg)	MS	MS	R	MS	MS	MS	MS	MS
Ampicillin (10 μg)	S	S	S	S	S	S	S	S
Cephalosporins								
Cephradine (30 μg)	R	S	R	MS	S	R	R	MS
Cefuroxime (30 μg)	S	S	S	S	MS	S	R	S
Inhibitors of protein synthesis								
Aminoglycosides								
Amikacin (30 μg)	S	S	S	S	S	S	S	S
Gentamycin (10 μg)	R	R	R	R	R	S	R	R
Streptomycin (10 μg)	S	S	S	MS	S	S	MS	MS
Tetracyclines								
Chloramphenicol (30 μg)	S	S	S	S	S	S	S	S
Tetracyclin (30 μg)	S	S	S	S	S	S	S	S
Macrolides								
Erythromycin (15 μg)	S	S	S	S	S	S	S	S
Inhibitors of nucleic acid synthesis								
Co- trimoxazole (25 μg)	S	S	S	S	S	S	S	S
Ciprofloxacin (5 μg)	R	R	R	R	R	R	R	R
Nalidixic acid (30 μg)	R	R	R	R	R	R	R	R
Norfloxacin (10 μg)	R	R	R	R	R	R	R	R
Inhibitors of cytoplasmic membrane functions								
Colistin (10 μg)	R	R	R	R	R	R	R	R
Urinary tract antiseptics								
Nitrofurantoin (300 μg)	S	S	S	S	S	R	S	S

S- Sensitive MS- Moderately sensitive R- Resistant.

Table 4. Bile salt hydrolase (BSH), cholesterol assimilation, β-galactosidase and hemolysis activities of the different isolates.

Isolate No.	BSH activity	Cholesterol assimilation (%)* Mean ± S.D	β-galactosidase activity* (Miller Units) Mean ± S.D	Hemolysis
JJ 18	+	73±2.1	843±7	−
JJ 22	+	46±2.1	824±5	−
JJ 24	+	56±1.5	914±10	−
JJ 29	+	67±1.0	518±7	−
JJ 30	+	68±1.2	322±5	−
JJ 55	+	41±1.0	868±12	−
JJ 58	+	46±2.1	1000±7	−
JJ 60	+	65±2.6	812±11	−

*The values are Mean ± SD of three independent experiments performed in duplicates.

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