Journal of Science and Technology ***54*** *(5) (2016) 632-642*

DOI: 10.15625/0866-708X/54/5/7562

PROBITOIC PROPERTIES OF *LACTOBACILLUS* STRAINS FROM FERMENTED FOOD AND INFANT FEACES

Le Nguyen Thi My[1], Huong Nguyen Thuy[2]

[1]*Department of Fishery, HCMC University of Food Industry, 140 Le Trong Tan Street, Tay Thanh Ward, Tan Phu District, Ho Chi Minh City, Vietnam*

[2]*Department of Chemical Engineering, HCMC University of Technology, 268 Ly Thuong Kiet, Ward 14, District 10, Ho Chi Minh City, Vietnam*

[*]Email*:* mylethang81@yahoo.com

Received: 22 December 2015; Accepted for publication: 2 April 2016

ABSTRACT

Lactobacillus strains are a major part of the probiotics, microflora of the intestine and of fermented foods. The aim of this study was to evaluate the potential probiotics of six *Lactobacillus* strains (*L. fermentum* 39-183; *L. plantarum* subsp.*plantarum* P-8; *L. casei* ATCC 334; *L. rhamnosus* ATCC 8530, *L. brevis* KB 290 and *L. fermentum* JMC 7776). Probiotic properties such as acid tolerance, bile resistance, bacteriocin-like activity, cell surface hydrophobicity and antibiotic resistance were assessed. *In vitro* results obtained showed that all *Lactobacillus* strains tested were able to meet the basic requirements for probiotic functions as they demonstrated probiotic characteristics such as tolerance to pH 2.0 and 2% bile salt. All *Lactobacillus* strains inhibited the growth of *E. coli*, *Staphylococcus aureus* and *Salmonella* Typhi. Among strains tested, *L. plantarum* subsp.*plantarum* P-8 showing inhibitory is very promising with inhibition zone ranging between 6.5 to 12.7 mm. The results for cell surface hydrophobicity and susceptibility against antibiotics also showed that *L. fermentum* JMC 7776 and *L. plantarum* subsp.*plantarum* P-8 had higher cell surface hydrophobicity than the rests. All *Lactobacillus* tested were resistant to vancomycin and susceptible to streptomycin. The results obtained in this investigation will be used to select potentially probiotic strains for *in vivo* study.

Keywords: Lactobacillus, probiotic, acid and bile tolerance, antibiotic susceptibility, bacteriocin-like activity.

1. INTRODUCTION

Probiotics are defined as living microorganisms that contribute to beneficial effects on human health upon ingested in adequate dose [1]. Recent research has credited several health benefits to probiotic organism that are indigenous to the gastrointestinal tract, as well as consumed through probiotic products. These include their ability to relieve symptoms of lactose intolerance [2], increase immune function cholesterol lowering potential [3], and treatment of diarrhea [4]. Some of the commonly known probiotics belong to the lactobacilli and bifidobacteria genus. Lactobacilli are members of the lactic acid bacteria (LAB). They are the largest genus in the LAB group with over 100 species reported. The natural habitats of Lactobacilli span from dairy products, sourdough breads, and fermented foods to various niches in animals and humans. Lactobacilli are part of human's normal microflora in small intestine, and large intestine [5].

Lactobacillus plays an important role as starters in health fermented foods. Some health benefits include improvement in intestinal disorders and lactose intolerance, altered vitamin content of milk, antagonism against various pathogenic organisms including antimutagenic and anti-carcinogenic activities [6, 7]. To be functional as probiotics for human, *Lactobacillus* must be of human origin, non-pathogenic, survive to gastric acid and bile toxicity, able to have cell surface hydrophobicity, colonise gastrointestinal tract (GIT) and able to compete with pathogen, as well as having ability to modulate immune responses. The antibiotic resistance of pathogenic bacteria is an increasing medical problem [8], and raises the question of antibiotic resistance among desired probiotic strains. Therefore, the antibiotic susceptibility test should be incorporated for the safety assessment of the desired property of the promising probiotic *Lactobacillus* [9]. Although *Lactobacillus* shows a high impact on effective protection to human health, there is obvious evidence that *Lactobacillus* from different origins possess probiotic properties at different levels [10]. Hence, the aim of this study is an effort to give a comparative account of six strains of *Lactobacillus* in the group of probiotic bacteria.

2. MATERIALS AND METHODS

2.1. Bacterial strains and culture conditions

The strains of *Lactobacillus* were isolated from two different origins: (*i*) traditional fermented food (*L. fermentum* 39-183; *L. plantarum* subsp.*plantarum* P-8; *L. casei* ATCC 334; *L. rhamnosus* ATCC 8530 and *L. brevis* KB 290) [11], (*ii*) fecal flora of infants (*L. fermentum* JMC 7776, accession number AB911502.1). The method of isolation was according to Schillinger (1999) using de Man Rogosa Sharpe (MRS) agar or broth (Merck Darmstadt, Germany) as a medium [12]. All isolated strains were kept at -20^{0}C in MRS broth supplemented with 50 % sterile glycerol for further experiments.

The pathogenic bacteria strains used as indicator for antimicrobial activity studies were *Escherichia coli* BL21, *Staphylococcus aureus* and *Salmonella* Typhi. Three indicators were supplied by the Department Biotechnology of Ho Chi Minh City University of Technology in Vietnam. All three indicator strains were stored at -20 ^{0}C in Tryticase soy broth supplemented with 50 % sterile glycerol.

2.2. Determination of acid tolerance

This experiment was carried out according to the method described by Brashear *et al*. [13] with some modification. A suspension of overnight culture of *Lactobacillus* strains in MRS broth was centrifuged at 6,000 rpm for 15 min. The cell pellets were mixed with 0.1 M sodium phosphate buffer pH 2.0 and 3.0 to yield 10^8 - 10^9 cfu mL^{-1}. The contents of the culture were vortexed and 1 mL of culture from each tube was taken later at 1 and 2 hours of incubation at 37 °C. The growth was estimated after 24 hours of incubation using standard plate count technique [14].

2.3. Determination of bile tolerance

The ability of *Lactobacillus* cultures to grow on bile containing media was performed according to Chou and Weimer [15]. One milliliter (1 mL) of overnight healthy culture (10^8 - 10^9 cfu mL^{-1}) was inoculated into 9 mL MRS broth containing different concentration of bile salt (0.5; 1.0 and 2.0 %) and incubated at 37 °C for 2 h. One hundreds microliter (100 µL) of the isolates was platted into MRS agar and incubated at 37 °C. The growth was estimated after 24 hours of incubation using standard plate count technique [14].

2.4. Antimicrobial activity

The antimicrobial activity of *Lactobacillus* strains was determined by the method introduced by Barefoot and Klaenhammer [16] with some modification. *Escherichia coli* BL21 and *Salmonella* Typhi were used as Gram negative pathogenic indicators while *Staphylococcus aureus* was of Gram positive. A loop full of each of the *Lactobacillus* strains from the MRS agar slants was inoculated into tubes containing 10 mL of sterile MRS broth. These broth cultures were incubated at 37 ^{0}C for 48 h. After incubation, the cultures were centrifuged (8,000 rpm for 15 min at 4 ^{0}C) to obtain the Culture Free Supernatant (CFS). The pH of the CFSs was adjusted to pH 6.5 with 1M NaOH to exclude antimicrobial effects of organic acids. The inhibition activity was examined by means of the diameters of inhibition zones using the agar well diffusion method [17]. Briefly, 50µL of cell-free supernatants were placed into wells (6.0 mm in diameters) on the appropriate media agar plates seeded with indicator strains (final concentration 10^6cfu mL^{-1}). After 24 h of incubation time, the diameter of inhibition zone was measured and scored. The presentation of inhibition zone were not included in 6 mm diameter of well. The inhibition zone larger than 2 mm was scored positive.

2.5. Cell surface hydrophobicity

The *in-vitro* cell surface hydrophobicity was determined by the bacterial adherence to hydrocarbon assay modified from the methods of Rosenberg *et al*. [18]. Briefly, *Lactobacillus* strains were grown in MRS broth for 18 - 24 h at 37 ^{0}C under anaerobic conditions. After incubation, the cultures were centrifuged at 5,000 rpm for 15 min, washed twice and resuspended in K_2HPO_4 buffer (pH 6.5) to an optical density ($OD_{600\ nm}$) of 0.4 - 0.6 (A_0) measured spectrophotometric. A portion of 2 mL of xylene or toluene was added to 6 mL of bacteria suspension. The mixture was blended using a vortex mixer for 60 s. The tubes were allowed to stand at 37 ^{0}C for 30 min to separate the two phases. The aqueous phase was carefully removed and the $OD_{600\ nm}$ of the aqueous phase (A) was measured. Hydrophobicity was calculated from three replicates as the percentage decrease in the optical density of the initial aqueous bacterial suspension due to cells partitioning into hydrophobicity (%H) of *Lactobacillus* strains adhering to xylene, toluene was calculated using the equation:

$$\%\,H = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

2.6. Resistance to antibiotics

The antibiotic susceptibility of *Lactobacillus* strains was determined towards six antibiotics, namely, Vancomycin (30 µg), Trimethoprime (1.25 µg), Penicillin (10 Units), Amoxicillin (20 µg), Erythromycin (15 µg) and Streptomycin (10 µg) by the disc diffusion method. After incubation at 37 ^{0}C for 24 h, inhibition zone diameters were measured and the results were expressed in terms of resistance (R), intermediate susceptibility (I) and susceptibility (S), according to cut off levels proposed by NCCLS and Vlkova *et al*. [19, 20].

2.7. Statistical analysis

All experiments in the present study were carried out in triplicates and the results indicate their mean values. For statistical analysis, the standard errors of the means were calculated and the means were tested according to one-variable analysis of Statgraphics centurion XV for significant differences among the samples.

3. RESULTS AND DISCUSSION

3.1. Acid tolerance

One of the most important properties for a probiotic to provide health benefits is that it must be able to overcome physical and chemical barriers such as acid and bile in the gastrointestinal tract [21]. Microbial strains suitable for probiotic should be able to tolerate in acid media with pH between 1.5 and 3.0 for at least 90 min since it is the food transit time through the human [22]. Thus, in this study, the media of pH 2.0 and 3.0 was used to represent the extreme acid condition of human stomach as in the case of fasting period when the stomach is non-fasting, e.g. after meal, the gastric pH is usually raised up to 3.0 or more. The survival rates of six *Lactobacillus* strains under different pH values are shown in Table 1. After 2 h of exposure, the majority of the six *Lactobacillus* strains was highly tolerant and retained their viability under acidic conditions at pH 3.0. The residual counts were within a range of 5 and 7 log counts throughout the period of exposure to pH 3.0. The survival at pH 3.0 but not at pH 2.0 was promising for most of the strains. There was more variation in the tolerance of pH 2.0 and the highest resistance to acidic conditions was observed for *L. plantarum* subsp.*plantarum* P-8 and *L. fermentum* JMC 7776. In contrast, the lowest acid tolerance was observed for *L. rhamnosus* ATCC 8530 (30.26 %) after 2 h of incubation at pH 2.0. The survival rates of *L. plantarum* subsp.*plantarum* P-8 decreased from 9.81 ± 0.16 to 5.74 ± 0.47, while *L. rhamnosus* ATCC 8530 decreased from 8.87 ± 0.27 to 2.66 ± 0.46 log CFU mL^{-1} by the end of 2 h exposure to pH 2.0. This result is similar with a report of Dhewa et al., (2010) that *L. plantarum* survived well at low pH [23]. However, our results also are not in agreement with Karimi Torshiz *et al.* [24], who observed the survival percentage at pH 2.0 after 2 h for *L. rhamnosus* was 67.76 ± 2.66 %. The results (Table 1) indicate that those strains had low tolerance at pH 2.0 were able to tolerate a higher pH of 3.0. This shows that the best pH to select for strains with probiotic potential is pH 2.0 since it is at this level and not pH 3.0 that discrimination according to pH sensitivity could be achieved. According to Hutkins and Nannen [25], bacterial strains were considered as acid resistant when more than 10 % of cells survive under pH 2.0 for 90 minutes, suggesting that six *Lactobacillus* strains are acid tolerance. To survive on acid condition, bacterial strains physiologically have to regulate their cytoplasmic or intracellular pH at a near neutral by using a number of transporters. One of the vital transporters in LAB is *Proton-translocating* ATP*ase* that maintains pH homeostatis by means of pumping H^+ out of cells [25]. Bacterial cells unable to maintain a near neutral intracellular pH during growth at low extracellular pH may lose viability and cellular activity.

Table 1. Tolerance of six *Lactobacillus* strains (log CFU count) on exposure to different pH and incubation period at 37 °C.

Strains	**Incubation (hours)**	**pH**		**Controls**
		2.0	**3.0**	**pH 6.2**
L. fermentum 39-183	1.0	7.74 ± 0.65	7.78 ± 0.21	9.87 ± 0.9
	2.0	3.66 ± 0.41	6.45 ± 0.24	9.91 ± 0.61
L. brevis KB290	1.0	6.67 ± 0.56	6.36 ± 0.19	8.72 ± 0.28
	2.0	3.79 ± 0.34	5.37 ± 0.28	8.78 ± 0.35
L. fermentum JMC 7776	1.0	7.78 ± 0.52	7.78 ± 0.21	8.73 ± 0.59
	2.0	4.61 ± 0.62	6.45 ± 0.24	8.78 ± 0.10
L. plantarum subsp.*plantarum* P-8	1.0	8.73 ± 0.30	8.56 ± 0.46	9.73 ± 0.51
	2.0	5.74 ± 0.47	7.73 ± 0.36	9.81 ± 0.16
L. casei ATCC 334	1.0	5.68 ± 0.29	8.57 ± 0.53	8.76 ± 0.48
	2.0	3.78 ± 0.25	6.80 ± 0.46	8.81 ± 0.19
L. rhamnosus ATCC 8530	1.0	5.60 ± 0.26	7.78 ± 0.34	8.79 ± 0.27
	2.0	2.66 ± 0.46	6.49 ± 0.27	8.87 ± 0.27

± = standard error of mean

3.2. Bile tolerance

Table 2. Tolerance of *Lactobacillus* strains (log CFU count) on exposure to different bile salt concentration after 2 h incubation at 37 °C.

Strains	**Control**	**Bile salt concentration (%)**		
		0.5	**1.0**	**2.0**
L. fermentum 39-183	8.67 ± 0.15	7.29 ± 0.18	5.98 ± 0.21	3.56 ± 0.11
L. brevis KB290	8.66 ± 0.13	7.59 ± 0.15	6.18 ± 0.12	5.11 ± 0.11
L. fermentum JMC 7776	8.69 ± 0.17	6.56 ± 0.10	4.39 ± 0.16	1.75 ± 0.25
L. plantarum subsp.*plantarum* P-8	8.67 ± 0.11	8.00 ± 0.17	5.79 ± 0.10	4.45 ± 0.15
L. casei ATCC 334	8.71 ± 0.14	7.68 ± 0.13	6.47 ± 0.14	5.59 ± 0.10
L. rhamnosus ATCC 8530	8.69 ± 0.17	8.23 ± 0.11	6.06 ± 0.13	3.98 ± 0.23

Another barrier for bacterial growth in the digestive tract is bile salts. As a surface active compound, bile penetrates and reacts with lipophilic side of bacterial cytoplasmic membrane causing a damage of membrane structure [26]. Bile also affects the structure and function of large macromolecules such as DNA and proteins leads to the damage of molecule. In this study, viability of six Lactobacillus strains on 0.5; 1.0; and 2.0 (%) bile salts for 2 h was presented in Table 2. As shown in table 2, all Lactobacillus strains were good stable in bile-containing media at concentration 0.5% and showed viable cell reduction less than 49 % at concentration 1.0 %.

L. casei ATCC 334 showed the highest survival percentage (64.24 ± 0.66 %) with cell viability decreased from 8.71 ± 0.14 to 5.59 ± 1.00 log CFU mL^{-1}. This result is in agreement with Puniya et al. [27] who observed L. casei showed a good tolerance to high bile concentration. In contrast, the lowest bile tolerance was observed for L. fermentum JMC 7776 on bile-containing media at concentration 2.0 % with viable cell reduction about 80 %. However, the relevant physiological concentrations of human bile salts range from 0.3 to 0.5 % [8]. The concentration 0.3 % bile salts is considered as critical for resistant strains screening and the same level is critical for the human probiotics selection. Therefore, the findings of present study indicated that six Lactobacillus strains have good bile intolerance and are more tolerant to bile salts than Lactobacillus spp. and Lactococcus sp. of earlier investigations [23].

3.3. Bacteriocin-like activity

The ability to produce antimicrobial compounds against enteric pathogens is one of the important criteria for probiotic bacteria. In this experiment, the culture supernatants after pH neutralization of *Lactobacillus* strains were examined for antimicrobial activity against pathogenic bacteria *E. coli*, *S. aureus*, and *Salmonella* Typhi (Table 3).

Table 3. Antimicrobial activity in terms of zone of inhibition (mm) of culture supernatants after pH neutralization of *Lactobacillus* strains against standard pathogenic cultures.

Strains	**Inhibition zone (mm)**		
	***E. coli* BL21**	***S. aureus***	***Salmonella* Typhi**
L. fermentum 39-183	11.30 ± 0.45	5.80 ± 0.00	7.50 ± 0.15
L. brevis KB290	4.00 ± 0.00	6.1 ± 0.50	6.90 ± 0.05
L. fermentum JMC 7776	7.20 ± 1.04	7.80 ± 0.76	8.20 ± 0.35
*L. plantarum*subsp.*plantarum* P-8	12.70 ± 0.76	6.50 ± 0.5	7.50 ± 0.87
L. casei ATCC 334	4.20 ± 0.26	9.10 ± 0.17	10.01 ± 0.36
L. rhamnosus ATCC 8530	3.17 ± 0.31	5.07 ± 0.30	5.03 ± 0.25

It was found that all *Lactobacillus* strains used in this study have shown a direct antagonism against *S. aureus* and produced an inhibition halo of growth of between 5 to 9 mm. Meanwhile, *L. casei* strain ATCC 334 showed highest antagonistic activity against *S. aureus* with inhibition zone of 9.10 ± 0.17 mm. The inhibition zone of *L. casei* ATCC 334 reported here is lower than *Lactobacillus casei* reported by Tharmaraj and Shah [28]. With the *Salmonella* Typhi, *L. casei* ATCC 334 showed highest antagonistic activity with inhibition zone of 10.01 ± 0.36 mm. *L. plantarum* subsp.*plantarum* P-8 showing inhibitory activity against all test organisms are very promising with inhibition zone of between 6.5 to 12.7 mm, thereby emphasizing its probiotic characteristics, whereas *L. rhamnosus* ATCC 8530 showed weak zones of inhibition against all test organisms. Our results are in agreement with N. Murugalatha *et al*. [29] who observed the inhibitory effects of *L. plantarum* isolated from raw Cattle milk, whose free-cell supernatant pH 7.0 showed strong activity against *Staphylococcus aureus* with the zone of inhibition of 10 - 14 mm in diameter. Pathogenic inhibition by LAB has previously been reported due to the production of organic acids, H_2O_2, and bacteriocin [30]. The inhibitory effect of bacteriocins was assumed to be due to there was effect on bacterial cells which destroyed the basic molecular structure of cell proteins and bacteriocin form the pores in the membrane of sensitive cells and depleted the transmembrane potential and/or the pH gradient, resulting in the leakage of cellular materials [31].

3.4. Cell surface hydrophobicity

The adhering ability of *Lactobacillus* strains studied *in vitro* by calculating the reduction in absorbance of buffer containing cellular suspension indicated that there was a vast difference in the hydrophobicity. *L. fermentum* JMC 7776 isolated from fecal of infants revealed 59.58 % hydrophobicity in toluene, and 44.26 % in xylene, while *L. fermentum* 39-183 fermented traditional foods origin showed 25.01 % hydrophobicity in toluene, and 22.43 % in xylene (Table 4). Adherence of bacterial cells is usually related to cell surface characteristics. Cell surface hydrophobicity is a nonspecific interaction between microbial cells and host. Bacterial cells with a high hydrophobicity usually present strong interactions with mucosal cells. In our study, the higher value of cell surface hydrophobicity of *L. fermentum* JMC 7776 and *L. plantarum* subsp.*plantarum* P-8 in two different hydrocarbons xylene and toluene were obtained. The high values of hydrophobicity could be a sign of a greater capability of bacteria to adhere the epithelial cells as indicated by Rosenberg *et al.* [18].

The results obtained in the present study are in agreement with that of Vinderola *et al*. [32] who observed the low value of hydrophobicity for the strains of *L. casei* and *L. rhamnosus*, found ranged from 10.9 to 24.1 %, are not in agreement with Puniya *et al*. [27] who observed the highest hydrophobicity were for *L. casei* ranging from 36 % to 56 %. The hydrophobicity of *L. fermentum* JMC 7776 was higher when compared to other strains with ranging from 44.26 to 59.58.

Table 4. Hydrophobicity of *Lactobacillus* strains as determined in selected hydrocarbons.

Strains	Hydrophobicity in %	
	Toluen	Xylene
L. fermentum 39-183	25.01 ± 3.81	22.43 ± 2.75
L. brevis KB290	39.41 ± 4.37	51.02 ± 1.04
L. fermentum JMC 7776	59.58 ± 3.01	44.26 ± 2.10
L. plantarum subsp.plantarum P-8	55.27 ± 4.63	40.89 ± 3.91
L. casei ATCC 334	30.56 ± 2.67	31.74 ± 2.50
L. rhamnosus ATCC 8530	39.39 ± 4.10	29.28 ± 2.41

3.5. Resistance to antibiotics

Lactobacilli are increasing incorporated into foods and other nutraceutical products due to their established health benefits [33]. In probiotic application, viable bacterial cells are consumed in high daily dose and the safety of the applied strain is therefore of utmost importance. One of the safety assessments is that the probiotic should be inhibited by common antibiotics agents. In this study, the susceptibility to certain antimicrobial agents was compared among six strains of *Lactobacillus.* Results as shown in table 5 revealed that all *Lactobacillus* strains were resistance to vancomycin and susceptible to streptomycin (Table 5).

Table 5. Susceptibility of *Lactobacillus* strains against antibiotics.

Strains	**Diameter of inhibition zone in mm**				
	Van (30µg)	**Tm (1.25µg)**	**Pn (10Units)**	**Ery (15µg)**	**S (10µg)**
L. fermentum 39-183	R	R	R	S	S
L. brevis KB290	R	R	R	S	S
L. fermentum JMC 7776	R	R	R	S	S
L. plantarum subsp.*plantarum* P-8	R	S	S	S	S
L. casei ATCC 334	R	R	R	R	S
L. rhamnosus ATCC 8530	R	R	R	S	S

Van = vancomycin R = ≤ 14; I = 15 - 16; S = ≥ 17; S = streptomycin R = ≤ 11; I = 12 - 14; S = ≥ 15; Ery = erythromycin R = ≤ 13; I = 14-17; S = ≥18; Tm = Trimethoprime R = ≤ 10; I = 11-15; S = ≥ 16; Pn = Penicillin R = ≤ 28; I = 28 - 29; S = ≥ 29; S = ≥ 18; R = resistant; I = intermediate susceptible; S = susceptible.

Resistance to vancomycin is commonly found in the genus *Lactobacillus*. The high frequency of vancomycin resistance found among lactobacilli might not pose a problem as this type of vancomycin resistance is different from the inducide transferable mechanism observed in Enterococci [34]. For trimethoprime *L. plantarum* subsp.*plantarum* P-8 showed susceptibility, whereas rests were resistant to this drug. Trimethoprime inhibits the synthesis of folic acid which is necessary for the synthesis purines, essential substance in bacteria nucleic acid. Resistance of almost *Lactobacillus* strains tested except for *L. plantarum* subsp.*plantarum* P-8 to trimethoprime was considered to be due to a trimethoprime-insensitive dehydrofolate reductase [35]. The results to the protein synthesis inhibitor showed that *L. casei* ATCC 334 was resistant to erythromycin whereas rests were susceptible to this drug. Our results of erythromycin susceptibility and trimethoprime resistance were also in agreement with Coppola *et al*. [36] and Ammor *et al*. [37].

4. CONCLUSIONS

In conclusion, all *Lactobacillus* strains tested were able to meet the basic requirements for probiotic functions as their probiotic characteristics such as tolerance to pH 2.0 and 2 % bile salt were demonstrated. All *Lactobacillus* strains inhibited the growth of *E. coli, Staphylococcus aureus* and *Salmonella* Typhi. *L. fermentum* JMC 7776 and *L. plantarum* subsp.*plantarum* P-8 had higher cell surface hydrophobicity than the rests. Besides, these strains tested were resistant to vancomycin and susceptible to streptomycin. The results obtained in this investigation will be used for preliminary screening in order to identify potentially probiotic bacteria suitable for human or animal use.

REFERENCES

1. FAO/WHO - Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Report of a joint FAO/WHO Expert Consultation, 2001.

2. Marteau P. R. - Probiotics in clinical conditions, Clin. Rev. Allergy Immunol. **22** (2002) 255-273.

3. Ooi L. and Liong M. - Cholesterol-Lowering Effects of Probiotics and Prebiotics: A Review of *in Vivo* and *in Vitro* Findings, Int. J. Molecular Sci. **11** (2010) 2499-2522.

4. Guandilini S., Pensabene L., Zibri M. A., Dias J. A., Casali L. G., Hoeskstra H., Kolacek S., Massar K., Micetic-Turk D., Papadopoulou A., De Sousa J. S., Sandhu B., Szajewska H., and Weizmzn Z. - *Lactobacillus GG* administered in oral rehydration solution to childern with acute diarrhoea: A multicentre European trial, J. Paediatr. Gastroenterol. Nutr. **30** (2000) 54-60.

5. Reuter G. - Probiotics-possibilities and limitations of their application in food, animal feed and in pharmaceutical preparations for men and animals, Berliner und Münchener Tierärztliche Wochenschrift **114** (2001) 410-419.

6. Molin G., Jeppsson B., Ahrné S., Johansson M. L., Nobaek S, Ståhl M., and Bengmark S. - Numerical taxonomy of *Lactobacillus* spp. associated with healthy and diseased mucosa of the human intestines, J. Appl. Bacteriol. **74** (1993) 314-323.

7. Dicks L. M. T. and Botes M. - Probiotic lactic acid bacteria in the gastro-intestinal tract: health benefits, safety and mode of action, Beneficial Microbes **1** (2010) 11-29.

8. Dunne C., O'Mahony L., Murphy L., Tornton G. Morrissey, D. O'Halloran S., Feeney M., Flynn S., Fitzgerald G., Daly C., Kiely B., O'Sullivan G. C., Shanahan F., and Collins J. K. - In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings, Am. J. Clin. Nutr. **73** (2001) 386-392.

9. Klayraung S., Viernstein H., Sirithunyalug J., and Okonogi S. - Probiotic properties of Lactobacilli isolated from Thai traditional food, Scientia Pharmaceutic **76** (2008) 485-503.

10. Haller D., Colbus H., Ganzle M. G., Scherenbacher P., Bode C., Hammes W. P. - Metabolic and functional properties of lactic acid bacteria in the gastrointestinal ecosystem: a comparative in vitro study between bacteria of intestinal and fermented food origin, System Appl. Microbiol. **24** (2001) 218-226. [197Hdoi:10.1078/0723-2020-00023].

11. Nguyen Thi My Le, Nguyen Thuy Huong, Pham Viet Nam – Probitoic properties od Lactobacilli isolated from Vietnam traditional fermented foods, International Journal of Renewable Energy and Environment Engineering **3** (2015) 06-12.

12. Schillinger U. - Isolation and identification of lactobacilli from novel-type probiotic and mild yoghurts and their stability during refrigerated storage, Int. J. Food Microbiol. **47** (1999) 9-87.

13. Brashears M. M., Jaroni D., and Trimble J. - Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle, J. Food Protection **66** (2003) 355-363.

14. Awan J. A. and Rahman S. U. - Microbiology Manual, Unitech Communications, Faisalabad, Pakistan, 2005, pp. 49-51.

15. Chou L. S. and Weimer B. - Isolation and characterization of acid- and bile-tolerant isolates from strains of *Lactobacillus acidophilus*, J. Dairy. Sci. **82** (1999) 23-31.

16. Barefoot S. F., Klaenhammer T. R. - Purification and characterization of the *Lactobacillus acidophilus* bacteriocin, lactacin B, Antimicrob. Agents Ch. **26** (1984) 328-334

17. Batdorj B., Dalgalarrondo M., Choiset Y., Pedroche J., Metro F., Prevost H. - Purification and characterization of two bacteriocins produced by lactic acid bacteria isolated from Mongolian airag, J. Appl. Microbiology **101** (2006) 837-848.
18. Rosenberg M., Gutnick D., and Rosenberg E. - Adherence of bacteria to hydrocarbons: a simple method for measuring cell hydrophobicity, FEMS Microbiology Letters **9** (1980) 29-33.
19. National Committee for Clinical Laboratory Standards (NCCLS) - Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, Second edition: Approved Standard M31-A2, NCCLS, Wayne, PA, 2002, pp. 80.
20. Vlkova E., Trojanova I., Rada V. - Distribution of bifidobacteria in gastrointestinal tract of calves, Folia Microbiological **51** (2006) 325-32.
21. Gibson G. R., and Fuller R. - Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and probiotics for human use, J. Nutr.**130** (2000) 391-395.
22. Havenaar R., Ten Brink B., and Huisin't Veld J. H. J. - Selection of strains for Probiotic use, In: Probiotics. The Scientific Basis, R. Fuller (Ed.) (Chapman & Hall, London), 1992, pp. 209-222.
23. Dhewa T., Bajpai V., Saxena R. K., Pant S., Mishra V. - Selection of lactobacillus strains as potential probiotics on basis of in vitro attributes, Int. J. Prob. Preb. **5** (2010) 45-52.
24. [24]. Karimi Torshizi M.A, Rahimi Sh, Mojgani N, Esmaeilkhanian S, Grimes J.L. - Screening of indigenous strains of lactic acid bacteria for development of a probiotic for poultry, Asian Australasian J. Animal Sci. **21**(2008) 1495-1500.
25. Hutkins R. W. and Nannen N. L. - pH homeostasis in Lactic Acid Bacteria, J. Dairy. Sci. **76** (1993) 2354-2365.
26. Succi M., Tremonte P., Raele A., Sorrentino E., Grazia L., Pacifico S., Coppola R. - Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano cheese, FEMS Microbiol. Lett. **244** (2005) 129-137.
27. Puniya M., Sangu K. P. S., Bharadwaj A., Gupta D., Kumar S., Dhewa T., and Pant S. - Probiotic and functional attributes of *Lactobacillus* spp. isolated from human faeces, J. Res. Antimicrobiol. **1** (2012) 032-042.
28. Tharmaraj N., and Shah N. P. - Antimicrobial effects of probiotics against selected pathogenic and spoilage bacteria in cheese-based dips, Int. Food Res. J. **16** (2009) 261-276.
29. Murugalatha N., Mohamkumar A. - Characterization and antibacterial activity of bacteriocin producing *Lactobacillus* isolated from raw cattle milk sample, Int. J. Biology. **3** (2011) 128-143.
30. Silva M., Jacobus N. V., Deneke C., and Gorbach S. L. - Antimicrobial substance from a human *Lactobacillus* strain, Antimicrob. Agents Chemother. **31** (1987) 1231-1233.
31. Zsolt Z., Edina N., Agnes B., and Anna H. - Influence of growth medium on hydrogen peroxide and bacteriocin production of *Lactobacillus s*trains, Food Technol. Biotechno. **43** (2005) 219-225.
32. Vinderola C. G. and Reinheimer J. A. - Lactic acid starter and probiotic bacteria: A comparative *in vitro* study of probiotic characteristics and biological resistance, Food Research International **36** (2003) 895-904.

33. Saarela M., Mogensen G., Fonden R., Matto J., and Matilla S. T. - Probiotic bacteria: Safety, functional and technological properties, J. Biotechnol. **84** (2000) 197-215.
34. Klein G., Hallmann C., Casas I. A., Abad J., Louwers J., Reuter G. - Exclusion of vanA, vanB and vanC type glycopeptide resistance in strains of *Lactobacillus reuteri* and *Lactobacillus rhamnosus* used as probiotics by Polymerase Chain Reaction and hybridization methods, J. Appl. Microbiol. **89** (2000) 815-824. [21Hdoi:10.1046/j.1365-2672.2000.01187.x].
35. Huovinen P. - Trimethoprim resistance, Antimicrob Agents Chemother. **31** (1987) 145-1456.
36. Coppola R., Succi M., Tremonte P., Reale A., Salzano G., and Sorrentino E. - Antibiotic susceptibility of *L. rhamnosus* strains isolated from Parmigiano Reggiano cheese, Lait. **85** (2005) 193-204.
37. Ammor M. S., Florez A. B., Mayo B. - Antibiotic resistance in non-enterococcal lactic acid bacteriTa and bifidobacteria, Food Microbiol. **24** (2007) 24559-24570.