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Probiotic potential of lactobacillus strains isolated from fresh bee pollen

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ABSTRACT

The present work aimed to evaluate the probiotic potential of lactobacillus strains isolated from fresh bee pollen. Thirty three lactobacillus isolates were screened for antagonistic activity against six pathogenic bacteria. Ten of them were selected identified as lactobacillus plantarum and evaluated for resistance to acidic pH (2 and 3) and 0.3% bile salts, hydrophobicity and autoaggregation ability. Moreover, their safety was verified by testing haemolytic activity on human blood agar and antibiotic resistance. The results showed that all lactobacillus strains were effective against all indicator bacteria; all strains were able to maintain their viability after 3h exposure to pH 3 and 4h in the presence 0.3% of bile salts. While only five could survive with losses in cell viability after 3h exposure to pH 2. Most strains showed a high hydrophobicity and autoaggregation ability. All lactobacillus strains were resistant to ciprofloxacin, tobramycin, nalidixic acid and colistin. 50% of the strains were susceptible to chloramphenicol, Nitroxolin, penicillin G, Cefoxitin, pristinomycin, cefexim and 80% are susceptible tostreptomycin. No haemolysis was observed on blood agar. Five strains of Lactobacillus plantarum were selected as suitable candidates for industrial use.

Keywords: *Lactobacillus*, Probiotic, Survival, bee pollen, antibacterial activity

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INTRODUCTION

The term probiotic derived from Greek and means ‘for life’, Lilly and Stillwell was first in 1965 that used the term probiotic for describing substances which simulate the growth of other microorganisms **[1]**. World Health Organization (WHO) has defined probiotics as” live microorganisms which when administered in adequate amounts confer a health benefit on the host”. The majority of the microorganisms used as probiotics belong to the genus Bifidobacterium and Lactobacillus **[2,3]**. This latter is the largest of lactic acid bacteria genera, including a microarophelic, no spore-forming, Gram positive and catalase negative bacteria. Lactobacilli are commonly found in diverse environment such as dairy products, animal and human mucosal surfaces as well as in plants and soil **[4]**. Due to the increasing consumer’s awareness that diet and good health are linked, probiotic lactic acid bacteria attracted great attention for the health promoting proprieties of certain species, leading to growing the demand on probiotic functional foods.

Several characteristics are essential in the selection of potential probiotics**[5].** The microorganism must be non-pathogenic could survive in the GIT; tolerate the low pH in stomach and physiological concentrations of bile, should exhibit good surface hydrophobicity for colonization and Must present antagonistic activity against intestinal pathogens **[6]**.

Bee pollen have been known and used by human since antiquity for medicinal purpose; Chinese and Egyptian societies used pollen for its miraculous ability of rejuvenation and healing, the Romans and Greeks called it “the life-giving dust”.

In the literature few reports are available about lactic acid bacteria originated from pollen**[7, 8, 9]**.The present work aimed to evaluate the probiotic potential of lactobacillus strains isolated from fresh bee pollen grains through evaluating the antimicrobial activity against pathogenic bacteria, tolerance to low pH and bile, cell surface hydrophobicity and autoaggregation. Also, their safety was investigated by antibiotics resistance and haemolytic activity.

MATERIALS AND METHODS

Isolation of lactic acid bacteria

Lactic acid bacteria were isolated from a fresh bee pollengrains sampled from different regions in Algeria. 2g of each fresh pollen samples was added to 100mL of MRS broth; well mixed and incubated for 72h at 30°C, Appropriate decimal dilutions were prepared in sterile physiological saline water, aliquots of 100µL were spread on the surface of MRS agar plate .incubation was carried out in anaerobic conditions using candle jar at 30°C for 48h. Representative colonies of LAB obtained were selected randomly for appropriate dilution, purified by streaking on MRS agar plate. Only Gram positive, rodand catalase-negative bacteria were kept in MRS broth, and were identified to the genus level by physiological test: CO_2 production from glucose, growth at 45°C and 10°C, growth in MRS containing 6.5 or 18%Nacl,as well as growth in MRS with pH 4.4 and 9.6. For identification to species level, the carbohydrate fermentation profiles were investigated using API 50 CHL medium (BioMérieux,, France) according to the manufacture's instruction.

Antibacterial activity

Antibacterial activity of lactobacillus strains were assessed by the spot on the lawn method described by Fleming*et al*.**[10]**against 6 pathogenic bacteria, *Salmonella typhimurium* ATCC 13311, *Escherichia coli* ATCC 25922, *Citrobacterfreundii* ATCC 8090, *Staphylococcus aureus* ATCC 25923,*Bacillus cereus* ATCC 10876 and *Listeria innocua* CLIP 74915. Briefly, 2 µL of overnight culture in MRS broth of each strain were spot inoculated on the surface dried of MRS agar, incubated anaerobically at 30°c for 48h. The inoculated agar plates were then overlaid with 7mL of BHI soft agar (0.7% agar); which had been seeded with 1% fresh culture of pathogenic bacteria. After 24h incubation at 37°C, a clear zone around the colonies was considered as positive inhibition.

Survival under conditions simulating the human GI tract

Resistance to low pH

Resistance of lactobacillus strains to low pH conditions was evaluated according to the methods described by Maragkoudakis *et al*. **[11]**, and *Guo et al*.**[5]**with minor modifications. Cells of overnight culture of each lactobacilli strain were collected by centrifugation (3000*g*, 15 min). The pellets were washed twice and finally suspended in PBS buffer solution pH 7.2. One mL aliquot of each suspension was added to 9 mL sterilized PBS solution that the pH was adjusted to pH 2 and 3 by 4 N HCl respectively. The suspensions were vortexed rigorously for 10 s and then incubated at 37°C for 3h,1 mL from each pH solution was serially diluted with sterile saline(0.85%). Appropriate dilutions were spread-plated onto MRS agar and incubated under anaerobic conditions at 37°C for 72h. The colony forming units were then estimated.

Bile salt tolerance

Bile salts tolerance was assessed by the method described by Tulumoglu *et al*.**[12]**and Argyri *et al*.**[13]**.Cells of overnight culture of each lactobacilli strain were collected by centrifugation (3000g, 15 min). The pellets were washed twice and finally suspended in PBS buffer solution pH 7.2. One mL aliquot of each suspension was added to 9 mL sterilized PBS solution supplemented with 0.3% (w/v) bile salts and incubated at 37°C for 4h. Bile tolerance was determined in terms of cell survival counts, as previously described.

Cell surface hydrophobicity

The cell surface hydrophobicity was measured according to the method of Rosenberg *et al*.**[14]**. Briefly, the test bacteria were grown in MRS broth at 30 °C under anaerobic conditions for 18–24 h, cells was harvested after centrifugation at 3 000 g for 15 min, washed twice and resuspended in 50mM K_2HPO_4 buffer (pH 6.5) to an optical density of 0.8-1.0 at 560 nm (A0). 0.6 mL of toluene was added to3 mL of bacterial suspension. The mixture was vortexed for 120sec. The tubes were allowed to stand at 37°C for 30 min to separate the two phases. The aqueous phase was carefully removed and the OD was measured at 560nm.

Hydrophobicity was calculated from tow replicates as the percentage decrease in the optical density of the initial aqueous bacterial suspension due to cells partitioning into a hydrocarbon layer. The percentage of cell surface hydrophobicity(H %) of the strain adhering to toluene was calculated using the equation:

$$H\ (\%) = [(A0 - A)/A0) \times 100].$$

Auto-aggregation

The auto aggregation assay was performed according to the method by Xu *et al*. **[15]**. with modifications. Fresh cultures of bacterial strain were harvested at 4000g for 10 min at room temperature. The cell pellet was washed twice with PBS and resuspended in the same buffer to an optical density of 0.5 ± 0.02 at 600 nm (A 0h). Each bacterial suspension (3 mL) was vortexed for 10s and incubated at 37°C for 2h. After incubation, the absorbance of the supernatant suspension was measured at 600 nm (A 2h). The autoaggregation percentage was expressed as

Auto-aggregation % = 1 - (A 2h/A 0h) x 100.

Antibiotic susceptibility

The antibiotic resistance of lactobacilli was evaluated using the agar disc diffusion method on MRS agar plates following the recommendation of the Committee of the antibiogram of French Society of Microbiology (2013).All isolates were screened for their susceptibility to Chloramphenicol, streptomycin (S) 10µI, nitroxolin (NO)30µg, cefotaxim(CTX) 30ug, penicillinG(P) 10µI, pristinomycin (RP) 15 µg, cefexim (CFX) 5µg, ciprofloxacin(CIP) 5µg , tobramycin (TOB) 10µg, nalidixic acid (NA) 30µg and colistin (CL)25µg. Results was expressed as Sensitive (S) or resistant (R) according to the standards of the Committee of the antibiogram of French Society of Microbiology (2013).

Haemolytic activity

Fresh lactobacilli cultures were streaked on TSA agar plates, containing 5% (w/v) human blood, and incubated for 48 h at 30 °C. Blood agar plates were examined for signs of β-haemolysis (clear zones around colonies), α-haemolysis (green-hued zones around colonies) or γ-haemolysis (no zones around colonies).

RESULTS

A total of 50 lactic acid bacteria (Gram positive, catalase negative) were isolated from fresh bee pollen grains, 33 isolates with rod shape were characterized and identified as belonging to lactobacillus genus; they were all homofermentative, grew at 10 and 45°C, in the presence of 6.5% Nacl, at pH 4.4. Lactobacillus isolates were screened for antibacterial activity towards pathogenic bacteria.

Antibacterial activity

AllLactobacillus strainsscreened for antagonistic activity by a spot on the lawn assay, were effective against both Gram-positive and Gram-negative pathogenic bacteria. The diameter of inhibition zones varied from 15.5mm to 48mm, the best inhibition zone was 48mm observed against *Salmonella typhimurium.*

Staphylococcus aureus was the most sensitive to the inhibitory action of lactobacillus followed by *Salmonella typhimurium* and *Citrobacterfreundii. Listeria innocua* was found to be the most resistant. *Bacillus cereus*and *Escherichia coli* have presented the same level of sensitivity**(Figure 1)**

The antagonistic activity exerted by lactobacillus strains was greater against Gram negative bacteria, than the Gram positive bacteria. Ten lactobacillus strains showing best results of antagonistic activity were selected**(table1)**, identified using the API 50 CHL system (bioMérieux, France), as *Lactobacillus plantarum***(table 2)**and subjected to evaluate their probiotic potential.

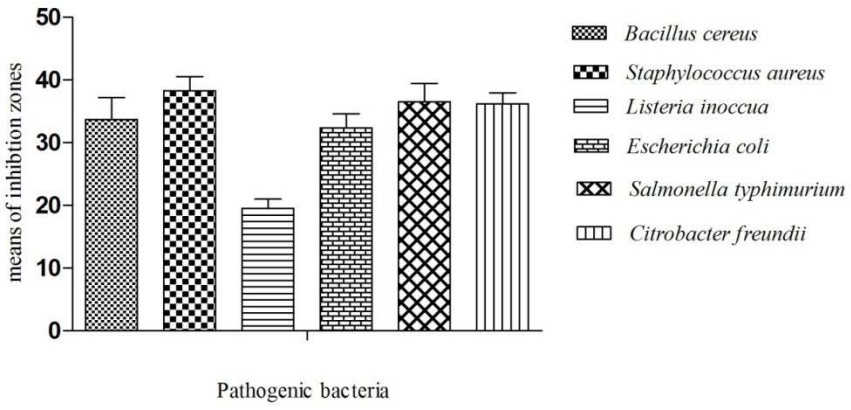

Figure.1.Antagonistic activity of lactobacillus strains against pathogenic bacteria
The mean of the diameters of inhibition zones of all strains ±SD (mm)

Table.1. Antibacterial activity of ten lactobacillus strains

	Bacillus cereus	*Staphylococcus aureus*	*Listeria inoccua*	*Escherichia coli*	*Salmonella typhimurium*	*Citrobacter freundii*
LB5	40 ± 00	42±2,82	22±00	31±1,41	37±00	39,5±0,7
LB8	34,5±2,12	40±2,82	21±2,82	30±1,41	41±1,41	39±00
LB11	41± 4,24	39±1,41	21±0,70	30±1,41	40,5±6,36	37±4,24
LB12	37 ± 2,82	40±2,82	22±1,41	32±0,70	35,5±6,36	34±2,82
LB15	38 ± 2,82	41±0,70	19±2,12	35±00	39±4,24	40±1,41
LB18	35 ± 7,07	41±2,82	24±2,12	32,5±3,53	38±2,82	33±00
LB22	36,5±4,94	40±1,41	19,5±2,12	33,5±0,70	34±8,48	36±00
LB27	36 ± 2,82	39±6,36	18,5±2,12	34±2,82	41±00	40±00
LB35	35 ± 7,07	43±0,70	23±00	33±4,24	33±1,41	40±1,41
LB45	35 ± 1,41	41±2,82	22±4,24	34,5±0,70	36±2,82	40±00

Table.2. Biochemical profile of the 10 Lactobacillus plantarum isolated from fresh bee pollen

Strains	**LB8**	**LB11**	**LB12**	**LB15**	**LB27**	**LB5**	**LB18**	**LB35**	**LB45**	**LB22**
Glycérol	–	–	–	±	±	–	–	–	–	–
Erythritol	–	–	–	–	–	–	–	–	–	–
D-Arabinose	–	–	–	–	–	–	–	–	–	–
L-Arabinose	–	–	–	–	–	–	–	–	–	–
D-Ribose	+	+	+	+	+	+	+	+	+	+
D-Xylose	–	–	–	–	–	–	–	–	–	–
L-Xylose	–	–	–	–	–	–	–	–	–	–
D-Adonitol	–	–	–	–	–	–	–	–	–	–
Méthyl-ßd-Xylopyranoside	–	–	–	–	–	–	–	–	–	–
D-Galactose	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+
L-Sorbose	–	–	–	–	–	–	–	–	–	–
L-Rhamnose	–	–	–	–	–	–	–	–	–	–
Dulcitol	–	–	–	–	–	–	–	–	–	–
Inositol	–	–	–	–	–	–	–	–	–	–
D-Mannitol	+	+	+	+	+	+	+	+	+	+
D-Sorbitol	–	–	–	–	–	–	–	–	–	–
Méthyl-Ad-Mannopyranoside	+	+	±	+	+	+	+	–	–	–
Méthyl-Ad-Glucopyranoside	–	–	–	–	–	–	–	–	–	–
N-Acétylglucosamine	+	+	+	+	+	+	+	+	+	+
Amygdaline	+	+	+	+	+	+	+	+	+	+
Arbutine	+	+	+	+	+	+	+	+	+	+
Esculine	+	–	+	+	+	–	+	+	+	+
Salicine	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+
D-Maltose	+	+	+	+	+	+	+	+	+	+
D-Lactose	+	+	+	+	+	+	+	+	+	+
D-Melibiose	+	+	+	+	+	+	+	+	+	+
D-Saccharose	+	+	+	+	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+	+	+	+	+
Inuline	–	–	–	–	–	–	–	–	–	–
D-Mélézitose	+	+	+	+	+	+	+	+	+	+
D-Raffinose	–	+	–	+	+	–	–	–	–	–
Amidon(Starch)	–	–	–	–	–	–	–	–	–	–
Glycogen	–	–	–	–	–	–	–	–	–	–
Xylitol	–	–	–	–	–	–	–	–	–	–
Gentiobiose	+	+	+	+	+	+	+	+	+	+
D-Turanose	+	+	+	+	+	+	+	+	+	+
D-Lyxose	–	–	–	–	–	–	–	–	–	–
D-Tagatose	–	–	–	–	–	–	–	–	–	–
D-Fucose	–	–	–	–	–	–	–	–	–	–
L-Fucose	–	–	–	–	–	–	–	–	–	–
D-Arabitol	–	±	–	±	±	–	–	–	–	–
L-Arabitol	–	–	–	–	–	–	–	–	–	–
Potassium Gluconate	±	+	–	+	+	–	±	–	–	–
Potassium 2-Cétogluconate	–	–	–	–	–	–	–	–	–	–
potassium 5-KetoGluconate	–	–	–	–	–	–	–	–	–	–
	L.plantarum	*L.plantarum*	*L.plantarum*	*L.plantarum*	*L.plantarum*	*L.plantarum*	*L.plantarum*	*L.plantarum*	*L.plantarum*	*L.plantarum*

Survival under conditions simulating the human GI tract

To survive the passage through the human gastrointestinal tract and exert their physiological activity, probiotics should be able to withstand the acidic environment in the stomach and bile salts in the beginning of the small intestine **[16]**.

Resistance to low pH

Lactobacillus strains isolated from fresh bee pollen were studied for their resistance to conditions of low pH 2 and 3.At pH 2 the viability of all strains was affected, after 3h exposure, only five strains could survive with losses in their viable count LB8, LB11, LB12, LB15 and LB27.While, the five other strains did not survive at all.The highest survival was observed for LB8 (6.30 log ufc/mL, 70.86%). At pH 3 all lactobacillus strains retained their viability **(Table3)**.

Table .3.Viability (log ufc/mL) of lactobacillus strains and survival percentage at low pH and in the presence of 0.3% bile

strains	Initial count (log ufc/mL)	pH 2.0 (log ufc/mL)	% survival [a]	pH 3.0 (log ufc/mL)	% survival [a]	0.3% bile (log ufc/mL)	% survival [a]
LB5	8,76	0	0%	8.46	96.57%	8.04	91 .78%
LB8	8,89	6.30	70.86%	8.08	90.89%	7.62	85.71%
LB11	8.52	4.69	55.04%	8.18	96%	7.84	92.01%
LB12	8.65	4.52	52.25%	8.34	96.41%	8.32	96.18%
LB15	8.81	5 .06	57.43%	8.54	96.93%	7.22	81.95%
LB18	9.32	0	0%	9.26	99.35%	8.29	88.94%
LB22	9.26	0	0%	9.17	99.02%	7.81	84.34%
LB27	9.27	4.95	53.33%	9.17	98.92%	8.07	87.05%
LB35	9.26	0	0%	8.98	96.97%	8.52	92%
LB45	9.49	0	0%	9.26	97.57%	8.06	84.93%

a:%survival= final (ufc/mL)/initial (ufc/mL) x 100.

Bile resistance

The results obtained showed that 0.3% bile salts didn't affect greatly all strains. The most tolerant was LB12 with 96.18% followed by LB11 92.01%, LB35 92% and LB5 91.78%.

Cell surface hydrophobicity

The hydrophobicity of bacterial surface can be a good indicator for screening potential probiotic strain**[15].**Results of cell surface hydrophobicity of the tested strains ganged from 18% to 85%, all strains showed high values of hydrophobicity expect LB15 which exhibited the lowest value(18%)**(Figure 2)**.

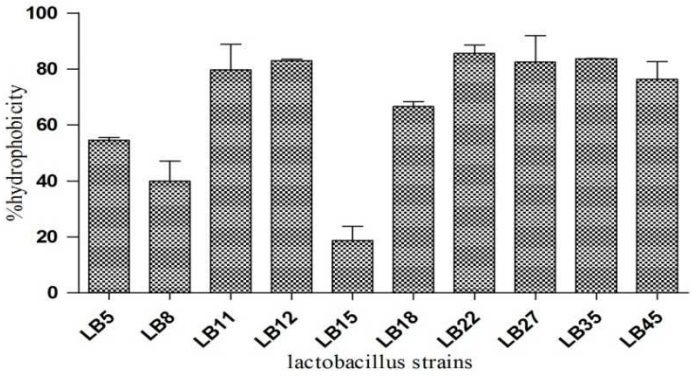

Figure.2. Hydrophobicity of lactobacillus strains

Autoaggregation

Lactobacillus strains showed autoaggregation values**(Figure 3)** ranging between 12.75% and 70.39%. LB27 showed the highest capability of autoaggregation among all the tested strains (70.39%), LB35 exhibited the lowest autoaggregation (12.75%) respectively.

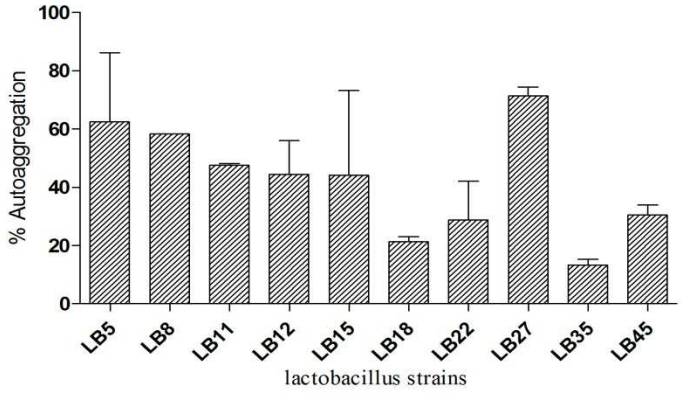

Figure.3. Autoaggregation of lactobacillus strains

Antibiotic susceptibility

All of *lactobacillus plantarum* strains was susceptible to chloramphenicol, Nitroxolin, penicillinG,Cefoxitin, pristinomycin, cefexim except LB15 ,LB18, LB27, LB45, LB22 and streptomycin except LB15, LB18, LB22.In contrast, all lactobacillus strains were resistant to ciprofloxacin, tobramycin, nalidixic acid and colistin**(table 4)**.

Table.4.Antibiotic susceptibility of lactobacillus strains

	C	NO	P	CFM	CTX	CIP	RP	TOB	NA	CL	S
LB5	S	S	S	S	S	R	S	R	R	R	S
LB8	S	S	S	S	S	R	S	R	R	R	S
LB11	S	S	S	S	S	R	S	R	R	R	S
LB12	S	S	S	S	S	R	S	R	R	R	S
LB15	S	S	S	R	S	R	S	R	R	R	R
LB18	S	S	S	R	S	R	S	R	R	R	R
LB22	S	S	S	I	S	R	S	R	R	R	R
LB27	S	S	S	R	S	R	S	R	R	R	S
LB35	S	S	S	S	S	R	S	R	R	R	/
LB45	S	S	S	I	S	R	S	R	R	R	S

Haemolytic activity

All strains were γ-haemolytic, no zones was observed around colonies .which indicate the absence of haemolysis activity for lactobacillus strains.

DISCUSSION

Numerous studies have addressed the possibility of use of lactic acid bacteria strains as bio- preservatives, owing to their inhibitory of food born and pathogenic microorganisms. Various factors may be involved in the antimicrobial activity of LAB. Among them, competition for substrates**[12]**, the diminution of pH due to the production of organic acids (lacticacid, acetic acid). In addition to the production of various compounds such as hydrogenperoxide (H_2O_2), diacetyl(2,3-butanedione)**[17]**, substances with a bactericidal or bacteriostatic action, including bacteriocins and bacteriocin-like substances**[12]**.

It is more known in the litterature that lactic acid bacteria are inhibitory for Gram positive bacteria more than Gram negative bacteria. This is due that the outer membrane of Gram negative bacteria contain many peptidoglycans which protect the cytoplasmic membrane from the action of antimicrobial agents **[17, 18]**. In this study the inhibitory activity of lactobacillus, was important towards the Gram negative bacteria. We suggest that it might be due to the action of Diacetyl whichisproduced by lactobacillus and is more active against the Gram negative bacteria, this compound react with the arginine-binding protein of gram-negative bacteria and thereby interfering with the utilization of this amino acid**[19]**.

Belhadj*et al.***[9]** found that the Gram-negative bacteria *E. coli, Salmonella typhimurium, Pseudomonas aeruginosa, and Shigella sp.* were strongly sensitive to the cell-free supernatants of *Lactobacillus plantarum, Pediococcus acidilactici and Pediococcus pentosaceus* isolated from raw Bee Pollen. Triaset *al.* **[20]**reported a good inhibitory activity of LAB originating from fruits and vegetables against spoilage pathogenic bacteria, suchas, *Listeria monocytogenes, Salmonella typhimurium and Escherichia coli.*

In the present study *listeria innocua* was found less sensitive to the antimicrobial action of lactobacillus. A greater sensitivity of *Listeria monocytogenes* towards some antibacterial compounds of lactic acid bacteria than *Listeria innocua* has been previously reported **[21, 22]**.

Resistance to conditions simulating human GI

The acidity of human stomach ranged from pH 1 to 4.5**[23, 11]**.Studying the survival of lactobacilli in simulated GI tract conditions*in vitro*, could give a prediction for the effective survival of the strain in *vivo* when consumed in a non-protected way**[24]**.All examined lactobacillus strains were resistant to pH 3 after 3 h exposure; these results are in agreement with pervious study**[11,5, 15]**.At pH 2 most strains display losses in their viability, the loss of viability of lactobacilli at this low pH was reported in several previous studies**[25, 5,12]**.Conway *et al*.**[26]**suggested that, the gastric juice may confer some protection to the strains comparing with the low pH buffer. Probiotic strains could be protected by food or other carrier matrix molecules, In order to avoid the exposition to the extremes of pH in the stomach **[2]**.

The suitable physiological concentrations of human bile, ranges from 0.3% to 0.5%**[27]**. A concentration of 0.3% is often considered to be critical concentration for selection of resistant strains **[5].** Lactobacilli strains examined in this study exhibited a good survival in the simulating small intestine environment; several authors report the same finding **[11.5,16]**. It was suggested that, bile-tolerant strains were suitable for alleviation of lactose intolerance symptoms **[16].**

Hydrophobicity and Autoaggregation

The ability to adhere to the intestinal mucosa is an important criterion for probiotic selection **[28, 15]**. Several mechanisms are involved in the adhesion of microorganisms to intestinal epithelial cells**[29]**.

Kaushik*et al*.**[30]**reported that hydrophobicity enables probiotics to bind and reside in the host intestines for a long time to deliver their beneficial effects. The determination of microbial adhesion to hydrocarbons is a way to estimate the ability of a strain to adhere to epithelial cells **[31]**.Vinderola*et al*. **[29]**found that hydrophobicity of 19 strains of lactobacillus from human origin varied from 14% to 53%. Almost, the same results of hydrophobicity are found by Ren*et al*. **[16]**with lactobacillus strains from fermented food and human intestine (14% -59%). Our results showed that all the lactobacillus strains present a high cell surface hydrophobicity expect LB15 which exhibited the lowest value (18%).Kaushik*et al*. **[30]**suggested that the differences in the cell surface hydrophobicity could be due to variation in the level of expression of cell surface proteins among strains of a species. Hydrophobicity and autoaggregation are considered as necessary traits for adhesion, they protect the host system by biofilm formation over the host tissue **[16]**. Strain LB27 exhibited a higher hydrophobicity and higher autoaggregation values 82% and 70% respectively this is in agreement with the finding of Ren*et al*. **[16]** with, *Lactobacillus salivarius subsp. Salicinius* and *Lactobacillusplantarum* showing higher hydrophobicity, autoaggregation and adhesion ability, which suggest their potential immunomodulatory activity in the GI tract.

Antibiotic susceptibility

Lactic acid bacteria widely used as probiotics or in starter cultures have the potential to serve as a host of antibiotic resistance genes with the risk of transferring the genes in many lactic acid bacteria and other pathogenic bacteria**[32]**.All lactobacillus strains were resistant to ciprofloxacin, tobramycin, nalidixic acid and colistin. Similar results are previouslyreported **[18,33]**. Various reports indicating that, lactic acid bacteria are normally resistant to principal types of antibiotics such as aminoglycosides, quinolone, fluoroquinolines**[34]**. Lactic acid bacteria are intrinsically resistant to antibiotics**[35,36]** and the intrinsic resistance is not horizontally transferable, and poses no risk in non-pathogenic bacteria **[32]**.All of *lactobacillus plantarum* strains were susceptible to chloramphenicol, Nitroxolin, penicillinG, Cefotaxim, pristinomycin andcefeximexpetLB15,LB18, LB27, LB45, LB22 and streptomycin except LB15, LB18, LB22.

Haemolytic activity

Absence of haemolytic activity is considered as a safety prerequisite for the selection of a probiotic strain**[37].** None of the strains exhibited β-haemolytic activity on TSA human blood agar. Similar results were previously reported by Maragkoudakis*et al*.**[11]**; Argyri*et al*. **[13]**.

Strains of *lactobacillus plantarum* LB8, LB11, LB12, and LB15and LB27 exhibited a potential antibacterial activity, good tolerance to acidic pH and bile, high values of hydrophobicity and autoaggregation.

CONCLUSION

In the present study, probiotic potential of ten *lactobacillus plantarum* strains from fresh bee pollen was investigated; five of them were found to possess desirable probiotic properties *in vitro*; Exhibited remarkable antimicrobial activity against pathogenic bacteria, a good tolerance to low pH and bile salts, a high hydrophobicity, and autoaggregation. These strains present good candidates for application as novel probiotic strains in the food industry.

REFERENCES

[1] S Ötleş. Probiotics and prebiotics in food nutrition and health, CRC Press, **2014**.
[2] J Prasad; H Gill; J Smart; PK Gopal.*International Dairy Journal*, **1998**, 8, 12, 993-1002.
[3]R Rubio; AJofré; B Martín; T Aymerich; M Garriga.*Food microbiology*, **2014**, 38, 303-311.
[4] S Lahtinen; AC Ouwehand; S Salminen; AV Wright. Lactic acid bacteria, microbiological and functional aspects, 4thed, CRC Press, **2012**.
[5] XH Guo, JM Kim, HM Nam, SY Park, JM Kim. *Anaerobe*, **2010**, 16, 4, 321-326.
[6] V Mishra; DN Prasad.*International Journal of Food Microbiology*, **2005**, 103, 1, 109-115.
[7] A Vásquez; TC Olofsson.*Journal of apicultural research*, **2009**, 48, 3, 189-195.
[8] H Belhadj; D Harzallah; S Khennouf; S Dahamna; S Bouharati; A Baghiani. *ActaHort (ISHS)*, **2010**, 854, 51-58.
[9] H Belhadj; D Harzallah; D Bouamra; S Khennouf; S Dahamna; M Ghadbane.*Bioscience of Microbiota, Food and Health*, **2014**, 33, 1, 11-23.
[10] HP Fleming; JL Etchells; RNCostilow.*Applied microbiology*, **1975**, 30,6, 1040-1042.
[11] PA Maragkoudakis ; G Zoumpopoulou ; C Miaris ; G Kalantzopoulos ; B Pot ; E Tsakalidou.*International Dairy Journal*, **2006**, 16, 3, 189-199.
[13] AA Argyri ; G Zoumpopoulou ; KAG Karatzas ; E Tsakalidou ; GJE Nychas;EZ Panagou ; CC Tassou.*Food Microbiology*, **2013**, 33, 2, 282-291.
[12] S Tulumoglu; ZN Yuksekdag; Y Beyatli; O Simsek ; B Cinar ; E Yaşar. *Anaerobe*, **2013**, 24, 36-42.
[14] M Rosenberg; D Gutnick; E Rosenberg.*FEMS Microbiology letters*, **1980**, 9, 1, 29-33.
[15] H Xu; HS Jeong; HY Lee; J Ahn.*Letters in applied microbiology*, **2009**, 49, 4, 434-442.
[16] D Ren ; C Li ; Y Qin ; R Yin ; S Du ; F Ye ; C Liu ; H Liu ; M Wang ; Y Li; Y Sun; X Li; M Tian ; N Jin.*Anaerobe*, **2014**, 30, 1-10.
[17] S Ammor ; G Tauveron ; E Dufour ; I Chevallier.*Food control*, **2006**, 17,6 ,454-461.
[18]O Ben Moussa; M Mankaï; KSetti; MBoulares; M Maher; M Hassouna.*Annals of microbiology*,**2008**, 58, 3, 461-469.
[19]SSalminen; AV Wright; AC Ouwehand; SLahtinen. Lactic acid bacteria: microbiological and functional aspects, 3rded,Marcel Dekker,New York,**2004**.
[20] R Trias; L Bañeras; EM Seguí; EB Romanyó.*International microbiology*, **2008**, 11,4, 231-236.
[21] AH Çon; HY Gökalp; M Kaya.*Meat science*, **2001**, 59, 4, 437-441.
[22] M Mataragas; EH Drosinos; J Metaxopoulos.*Food Microbiology*, **2003**, 20, 2, 259-265.
[23]WH Lin; CF Hwang; LW Chen; HY Tsen.*Food Microbiology*, **2006**, 23, 1, 74-81.
[24]Y Zhang; L Zhang; M Du; H Yi; C Guo; Y Tuo; XHan; J Li; L Zhang ; L Yang. *Microbiological research*, **2011**, 167, 1, 27-31.
[25] CC Tsai ; HY Hsiha ; HH Chiua ; YY Laib ; JH Liuc ; B Yua ; HY Tsend.*International journal of food microbiology*, **2005**, 102, 2, 185-194.
[26] PL Conway; SL Gorbach; BR Goldin.*Journal of dairy science*, **1987**, 70, 1, 1-12.
[27] C Dunne; L Murphy; S Flynn; L O'Mahony; S O'Halloran; M Feeney; D Morrissey; G Thornton; G Fitzgerald; C Daly; B Kiely; E M M Quigley; G C O'Sullivan; F Shanahan; J K Collins.*Antonie van Leeuwenhoek*, **1999**, 76, 279-292.
[28] EM Tuomola, SJ Salminen.*International journal of food microbiology*, **1998**, 41, 1, 45-51.
[29] G Vinderola ; B Capellini ; F Villarreal; V Suárez; A Quiberoni; J Reinheimer.*LWT-Food Science and Technology*, **2008**, 41, 9, 1678-1688.
[30] JK Kaushik; A Kumar; RK Duary; AKMohanty; S Grover; VK Batish.*PloS one*, **2009**, 4, 12.
[31] CG Vinderola; JA Reinheimer.*Food Research International*, **2003**, 36, 9, 895-904.
[32] S Mathur; R Singh.*International Journal of Food Microbiology*, **2005**, 105, 3, 281-295.
[33]M A Herreros; H Sandoval; L González; JM Castro; JM Fresno; METornadijo.*Food microbiology*, **2005**, 22, 5, 455-459.
[34] PM Halami; AChandrashekar; K Nand.*Letters in applied microbiology*, **2000**, 30, 3, 197-202.
[35] S Salminen; A v Wright; L Morelli; P Marteaud; D Brassart; W M de Vos; R Fondén; M Saxelin; K Collins; G Mogensen; SE Birkeland; TM Sandholm. *International journal of food microbiology*, **1998**, 44, 1, 93-106.

[36] D Chemlal-Kherraz; F Sahnouni; AMatallah-Boutiba; Z Boutiba.*African Journal of Biotechnology*, **2012**, 11, 68, 13220-13227.
[37]FAO/WHO, Guidelines for the evaluation of probiotics in food – JointFood and Agricultural Organization of the United Nations and World HealthOrganization Working Group Meeting Report, **2002**, London Ontario, Canada.