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Evaluation of *Lactobacillus acidophilus, Lactobacillus casei* and *Lactobacillus plantarum* for probiotic characteristics

[1]Soliman A. H. S.,[2] Sharoba A. M., [2]Bahlol H.E.M., [2]Soliman A. S. and [1]Radi O. M. M.

[1]Horticulture Crops Technology Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.
[2]Food Science Department, Faculty of Agriculture, Benha University, Egypt.

ABSTRACT

Lactobacillus strains are a major part of the probiotics, microflora of the intestine as well as of fermented dairy products, and are found in a variety of environments. The aim of this study was to find out the ability of bile salts and acid tolerance as well as antibacterial properties of the three lactobacillus strains namely *Lactobacillus acidophilus* ATCC 20552, *Lactobacillus casei* DSM 20011 and *Lactobacillus plantarum* ATCC 14917. The acid tolerance test was studied under pH 2.0 and 3.0 with 6.5 as a control. The cell count for the acid tolerance test was obtained at an interval of 0, 1, 2 and 3 hours, and was pour plated on Man Rogosa, and Sharpe (MRS) agar to be incubated at 37 °C for 24 hours. All cells were selected for bile tolerance test in MRS broth containing bile concentrations of 0 % as control and 0.1, 0.3, 0.5 and 0.7 % as test and antibiotic susceptibility. Results showed that the three strains of Lactobacilli have ability to tolerate acid and bile salts and resistance to 0.4 % of phenol and were susceptible for streptomycin (10 µg), ampicilline (10 µg), chloramphenicol (30 µg), erytromycin (15 µg), amoxicillin/clavulanic acid (30 µg) and rifampicin (5 µg). All strains have antimicrobial activity against seven indicator bacteria included *Bacillus cereus* DSM 351, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 12600, *Listeria monocytogenes, Salmonella typhimurium* ATCC14028, *Klebseilla pneumonia* ATCC 1705 and *Pseudomonas aeruginosa* ATCC 43495.

Key words: Probiotic, Lactobacillus, acid tolerance, bile salts tolerance, antibiotic susceptibility and antibacterial properties.

Introduction

Probiotics have been defined as “live microbial food supplements which beneficially affect the host by improving the intestinal microflora balance” or more broadly as “living microorganisms, which upon ingestion exert health benefits beyond inherent general nutrition”. There are, more recently, increasing experimental and clinical data to support the use of proven probiotic organisms in prevention and treatment of many gastrointestinal disorders (Manisagar, 2012).

Probiotic bacteria are increasingly used in food and pharmaceutical applications to balance disturbed intestinal microflora and related dysfunction of the human gastrointestinal tract. *Lactobacillus acidophilus* and *Bifidobacterium spp*. have been reported to be beneficial probiotic organisms that provide excellent therapeutic benefits. The biological activity of probiotic bacteria is due in part to their ability to attach to enterocytes. This inhibits the binding of enteric pathogens by a process of competitive exclusion. Attachment of probiotic bacteria to cell surface receptors of enterocytes also initiates signalling events that result in synthesis of cytokines. Probiotic bacteria also exert an influence on commensal micro-organisms by the production of lactic acid and bacteriocins (Kailasapathy and Chin, 2000). As probiotic bacteria, the lactic acid bacteria are confronted with several challenges, such as high acid concentration and high bile salt concentration. Acid tolerance is the principal characteristic of every strain that can survive and function in the alimentary canal (Morelli, 2000 and Barmpalia-Davis *et al*., 2008). The depressant effect of bile salts on the growth of bacteria has some relationship with the bile salts concentration and bacteria characteristics. The bacteria that can survive and metabolize in normal physio-bile salt concentrations may have the ability to survive in the transport process of the intestinal tract (Yeung *et al*., 2002). It is essential to separate bacteria that have high bile salt tolerance. Hence, whether they can survive in the acid and bile salt conditions in the human gastrointestinal tract is accepted as one of the desirable properties used to select potential probiotic strains (Gilliland and Walker 1990).

Bacteriocins are produced by some strains of lactic acid bacteria (LAB); they are antimicrobial peptides with activity against strains closely related to the producer microorganism. Some bacteriocins are also active against gram-positive food-borne pathogens such as *Listeria monocytogenes, Staphylococcus aureus, Bacillus*

Corresponding Author: Soliman A. H. S., Horticulture Crops Technology Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

subtilis and spores of *Clostridium perfringens*. For this reason, they have received much attention for using as natural or so-called 'biopreservatives' in food in recent years (Savadogo *et al.,* 2004).

The objective of the present study was to collect different lactic acid bacterial strains from culture collection center and screen their functional probiotic characteristics such as acid tolerance, bile tolerance, phenol resistance, antibiotic sensitivity and antibacterial activity for their commercial use.

Materials and Methods

Bacterial strains

Strains were obtained from Egyptian Microbial Culture Collection, (EMCC), Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Three strains of Lactobacillus *strains* used for comparative studies; i.e. *Lactobacillus acidophilus* ATCC 20552, *Lactobacillus casei* DSM 20011 and *Lactobacillus plantarum* ATCC 14917. They were maintained by subculturing on MRS agar (meat peptone 10 g/l, dextrose20 g/l, yeast extract 5g/l, beaf extract 10 g/l, disodium phosphate 2 g/l, sodium acetate 5 g/l, ammonium citrate 2 g/l, magnesium sulfate 0.1 g/l, manganese sulphate 0.05 g/l, tween 80 1g/l and agar 15 g/l) according to Oxoid-manual (1998). Test microorganisms viz. *Bacillus cereus* DSM 351, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 12600*, Listeria monocytogenes, Salmonella typhimurium* ATCC14028*, Klebseilla pneumonia* ATCC 1705 and *Pseudomonas aeruginosa* ATCC 43495 were assessed for experiments of antimicrobial activity. They were maintained on trypton glucose extract agar (beef extract 3g/l, tryptone 5 g/l glucose 1 g/l and 15 g/l agar) according to APHA-manual (1992). The stock cultures were preserved in 10% glycerol and were subcultured routinely at the interval of every two months. The cultures were stored at 4°C between transfers and were subcultured once before experimental use.

Enumeration of probiotic microorganisms

The viability of probiotic cultures was determined and expressed as colony forming units (CFU/mL) on MRS agar. Serial dilutions were prepared in sterile physiological solution before plating on to MRS agar. The plates were incubated at 37 °C for 24 h.

Acid tolerance

Lactic acid bacteria cells grown in MRS broth were collected by centrifuging at 3000 rpm for 15 min. The cell pellet was washed twice and resuspended in 10 ml of phosphate-buffered saline (PBS) to obtain 10^9 cfu/ml before addition to sterile PBS with the pH values of 2, 3 and 6.5 (adjusted using 5 M HCl). Phosphate-buffered saline was prepared by dissolving NaCl (9 g/l), $Na_2HPO_4.2H_2O$ (9 g/l) and KH_2PO_4 (1.5 g/l) in distilled water. The tubes were incubated at 37^{o}C and the viable organisms were counted after exposure to acidic conditions for 0, 1, 2 and 3 h on MRS agar incubated at 37^{o}C for 48 hours. The survival cell count was calculated according to the number of colonies grown on MRS agar, compared to the initial bacterial concentration (Buntin *et al.,* 2008).

Bile salts tolerance

The growth rate of bacterial cultures was determined in MRS broth containing different levels (0, 0.1, 0.3, 0.5 and 0.7%) of bile salts (oxgall). Freshly prepared cultures were inoculated (1%) into MRS broth and incubated at 37°C for 24 h under aerobic condition. Optical densities were spectro-photometrically measured at 620 nm against the uninoculated broth after 0, 2, 4, 6, 8 and 24 h (Al- Saleh *et al.,* 2006).

Resistance to 0.4 % phenol

The ability of Lactobacillus strains to grow in the presence of phenol by inoculating cultures (1 % of an overnight culture) in MRS broth with and without 0.4 % phenol was tested. Serial dilutions were spread-plated onto MRS agar at 0 time and after 24 h of incubation at 37°C to enumerate surviving bacteria as described by Xanthopoulos *et al.* (2000).

Antibiotic resistance patterns

Disc diffusion method was used to determine the antibiotic resistance of the tested Lactobacillus strains. These antibiotic disc were followed as: (streptomycine (10 µg), ampicilline (10 µg), kanamycine (30 µg), clindamycine (2 µg), chloramphenicol (30 µg), erytromycin (15 µg), amoxicillin/clavulanic acid (30 µg),

rifampicin (5 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), vancomycin (30 µg) and gentamycin (10 µg)) from Oxoid, Italy. *Lactobacillus* strains were grown in MRS broth overnight. Following the preparation of 10^5 cfu/ml dilutions of bacteria, freshly poured MRS plates were equally inoculated with this dilution. Antibiotic discs were placed on the inoculated plates using sterile forceps, and the plates were incubated at 37 ºC. A zone of inhibition was measured in 'mm' and the result interpreted on the basis of CLSI standards (Wayne, 2007).

Antimicrobial activity of probioticated juices test by disc diffusion method

Antibacterial activities were assayed using cell-free neutralized supernatants (CFNS). The CFNS were obtained from cultures grown in MRS broth at 37 ºC for 24 h. Cultures were centrifuged at 5000 rpm for 15 min and then supernatant pH was adjusted to be 6.5 with 1 M NaOH. The supernatant was then heated for 5 min at 80 ºC, cooled and followed by filtration through 0.2 mm Millex-GV membranes (Millipore). The neutralized supernatants were tested against indicator strains using paper disc assay as described by Bonev *et al.* (2008). Tryptone glucose agar plates with inoculated indicator bacteria used for agar disc diffusion method. Blank discs (6 mm diameter, 1 mm thickness) were immersed into cell free supernatant of the Lactobacillus strains for 15 min and were placed onto the agar surface. The plates were left at room temperature for 1 h so that the absorbed supernatant become diffused into agar, and then incubated at 37°C for 24 h.

Results and Discussion

Acid susceptibility

Probiotic bacteria are mostly delivered in a food system and must be acid and bile tolerant to survive in the human gastrointestinal tract. The pH in human stomach ranged from 1(during fasting) to 4.5 (after a meal) and food ingestion can take up to 3 h *(*Wang *et al.,* 2009). The effect of pH on *L. acidophilus, L. casei* and *L. plantarum* strains were tested and the number of viable cells for selecting the strains resistant to low pH and PBS, the pH was adjusted to 2 and 3 comparing to pH 6.5. The time taken during the digestion in the stomach is 3 h. So, all Lactobacillus strains were detected whether resistant to low pH during 3 h or not. Data illustrated in Fig. (1) shows that *L. acidophilus* was survived at pH 2 during an incubation period of 2 h and then the growth was delayed after 3 h comparing to control (pH 6.5). The number of log cfu ml^{-1} remains essentially steady and it is not differs to the control after 3 h of incubation.

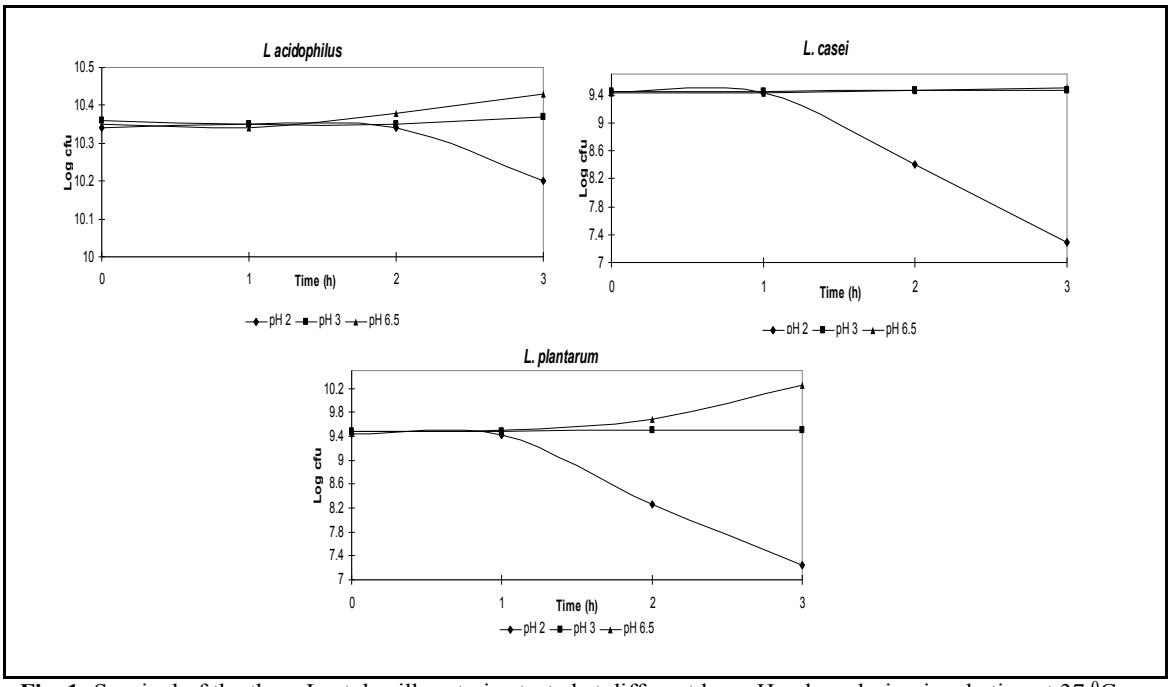

Fig. 1: Survival of the three Lactobacillus strains tested at different low pH values during incubation at 37 ^{0}C for 48 h.

Data in the same figure show the viability of *L. casei* and *L. plantarum*. No loss of viability was detected over 3 h of exposure to pH 3.0, indicating a naturally high level of acid resistance in *L. casei and L.*

plantarum to hydrochloric acid. In contrast, exposure to pH 2 eliminated more than 11.2 and 12.4 % for *L. casei* and *L. plantarum*, respectively during an incubation period of 2 h and then after 3 h elimination was more than 22.0 and 23.3 % for *L. casei* and *L. plantarum* , respectively at the same pH. Acid tolerance of Lactobacillus strains was also reported by Dixit *et al.* (2013) who recorded that, the survival of *L. acidophilus* at pH 2.5 after 4 h was very significant with high acid tolerance. Srinu *et al.* (2013) showed that all the selected lactic acid bacterial strains (*L. plantarum and L. casei*) were good survival abilities in the tested acidic pH range (1.5, 2.0, 3.0 and 3.5) and Shivram and Vishwanath (2012) reported that, the isolated *Lactobacillus* strains were tolerable to pH 2 and 3. Also, Mourad and Nour-Eddine, (2006) reported that *L. plantarum* was survived at an incubation periods of 2 to 6 h at pH 2.0 and 3.0. Park *et al.* (2006) compared the pH resistance of the four strains of *L. acidophilus* at pH 2, 3, 4, 5 and 7. *Lactobacillus acidophilus* isolated from rat and chicken showed little or no decrease in viable cell numbers for up to 240 min at pH 3, 4, 5 and 7. Jamaly *et al.* (2011) found *L. plantarum* able to tolerate 3 h of acid exposure with pH 2.0 and 3.0.

Bile salt tolerance

Bile is a result from a digestive secretion that can play a capital role in lipids emulsification and has the ability to affect the phospholipids, cell membrane proteins and disrupt cellular homeostasis (Burns *et al.,* 2008). Bile tolerance is one of the most crucial properties as it determines the ability of bacteria to survive in the small intestine, and consequently their capacity to play their functional role as probiotics. The ability of Lactobacillus strains to grow in the presence of bile salts was studied by growing them in MRS broth supplemented with different concentrations of bile salts (0.1 to 0.7%). Bile tolerance is an important characteristic of probiotic microorganisms. A concentration of 0.3% of bile salts closely appropriates the bile level found in the gastrointestinal tract (Goldin and Gorbach 1992). Results from comparison of different cultures for bile salts tolerance are shown in Table (1). All strains exhibited considerable variations with regard to growth in control broth after 24 h. The optical densities of *L acidophilus* reached to 2.905, 2.900, 2.721, 2.740 and 2.626 at concentration 0, 0.1, 0.3, 0.5 and 0.7 % of bile salts, respectively. While, the corresponding percentage were 2.882, 2.748, 2.668, 2.573 and 2.476 for *L. casei* and it reached 2.905, 2.822, 2.314, 2.250 and 2.229 for *L. plantarum* after 24 h.

Table 1: Effect of bile salt concentration on growth of Lactobacillus strains at 37^0C.

Strains	Bile salt concentration %	Time (h)						Surviving (%) after 24 h
		0	2	4	6	8	24	
L. acidophilus	0.0	0.095	0.186	0.522	1.325	2.014	2.905	100.00
	0.1	0.102	0.162	0.439	1.140	1.860	2.900	99.82
	0.3	0.116	0.187	0.389	0.797	1.463	2.721	93.66
	0.5	0.021	0.092	0.294	0.702	1.368	2.740	94.32
	0.7	0.118	0.182	0.349	0.612	1.262	2.626	90.39
L. casei	0.0	0.138	0.227	1.282	2.317	2.803	2.882	100.00
	0.1	0.153	0.269	1.333	2.272	2.426	2.748	95.35
	0.3	0.170	0.272	1.215	1.931	2.115	2.668	92.57
	0.5	0.167	0.286	1.061	1.855	2.058	2.573	89.27
	0.7	0.164	0.336	1.156	2.015	2.180	2.476	85.91
L. plantarumi	0.0	0.039	0.090	0.265	0.758	1.627	2.905	100.00
	0.1	0.053	0.105	0.278	0.716	1.487	2.822	97.14
	0.3	0.074	0.097	0.246	0.584	1.149	2.314	79.65
	0.5	0.075	0.128	0.232	0.492	0.923	2.250	77.45
	0.7	0.060	0.099	0.242	0.497	0.848	2.229	76.72

Surviving percentage (%) = (OD of bile salt /OD of control) x100

In another way, the obtained results also showed that three strains were observed resistant bile salt, corresponding to survival percentages ranging from 99.82, 93.66, 94.39 and 90.32 %, respectively, after 24 h incubation for *L. acidophilus* and resistance percentage of *L. casei* was 95.35, 92.57, 89.27 and 85.91 % at 0.1, 0.3, 0.5 and 0.7 % of bile salt, respectively after 24 h incubation. While, *L. plantarum* was also resists to bile salt and it was 97.14, 79.65, 77.45 and 76.72 % at 0.1, 0.3, 0.5 and 0.7 % of bile salt, respectively, after 24 h incubation. These results are approach with those of Jamaly *et al.* (2011), they reported that 10 out of 18 resistant Lactobacilli strains to 0.3% Ox-bile (with resistance % $\geq$ 50) were obtained and identified as *L. plantarum*, *L. paracasei* and *L. brevis*. Kim *et al.* (2006) mentioned that *L. gasseri, L. acidophilus and L brevis* grew in the presence of bile at 1.0%, during incubation for 24 h. The survival of Lactobacillus strains at bile concentrations of 0.5 and 1% were very similar. *Lactobacillus gasseri* showed more resistant than other strains. In the presence of 1% bile salts, *L. gasseri* was increased by 2.11 log cfu/ml for 24 h incubation. On the other hand, *L. acidophilus* showed a high degree of sensitivity, and was increased by 1.12 log cfu/ml with bile salts, while by 2.94 log cfu/ml without bile salts. Conway *et al.* (1987) suggested that, these differences in acid and bile tolerance of strains from species might be due to difference in the cell wall structure. Ruiz *et al.* (2013) reported that Lactobacillus and Bifidobacterium display a variety of proteins devoted to the efflux of bile salts or

protons, to modify sugar metabolism or to prevent protein misfolding. Boke *et al.* (2010) suggested that, the exopolysaccharides produced by lactic acid bacteria are thought to play a role in the protection of microbial cells against low pH and bile salts.

Resistance to 0.4% phenol

Most strains grow, although at lower levels, in the presence of phenol at 0.4 % during incubation for 24 h (Table 2). Phenols may be formed in the intestine as a result of bacterial deamination of some aromatic amino acids derived from dietary and endogenous proteins. These compounds are known to have bacteriostatic properties at least in vitro (Suskovic *et al.,* 1997). Results indicate a different resistance of Lactobacillus strains tested. In general, there is a good tolerance of all tested strains towards phenol even if the growth in presence of phenol was lower than in MRS broth without phenol.

Table 2: Ability of *Lactobacillus* strains to grow in the presence of 0.4% phenol at 37^0C.

Strains	Viable counts (log10 cfu ml^{-1})					
	MRS broth			MRS broth + 0.4% phenol		
	Zero time	24 h	Increase (%)	Zero time	24 h	Increase (%)
L. acidophilus	10.33	11.49	11.22	10.34	10.55	2.03
L. casei	9.45	10.74	13.65	9.43	9.65	2.33
L. plantarum	9.42	10.98	16.56	9.40	9.62	2.34

Increase = [(log_{10} (final population) -log_{10} (initial population)] / log_{10} (initial population)) X100

Xanthopoulos *et al.* (2000) suggested that 0.4 % of phenol causes a bacteriostaic action in some microorganisms, and an increase in viable count was observed in the presence of phenol after 24 h for *L. acidophilus* DC 601 Raja *et al.* (2009) and Hoque *et al.* (2010) observed that isolated *Lactobacillus* spp. were resistance to inhibitory substances like phenol at 0.4%.

Antibiotic susceptibility

The determination of antibiotic susceptibility of a bacterial strain is an important prerequisite prior to considering it safe for human and animal consumption (Dixit *et al.,* 2013).

Results are summarized in Table (3), showed *Lactobacillus acidophilus* has shown sensitive to streptomycin (10 µg), ampicilline (10 µg), chloramphenicol (30 µg), erytromycin (15 µg), amoxicillin/ clavulanic acid (30 µg) and rifampicin (5 µg) and it has intermediate sensitive for clindamycine (2 µg) while, the inhibition zones were not observed for kanamycine (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), vancomycin (30 µg) and gentamycin (10 µg).

Lactobacillus casei was susceptible to streptomycin (10 µg), ampicilline (10 µg), chloramphenicol (30 µg), erytromycin (15 µg), rifampicin (5 µg), cefotaxime (30 µg) and gentamycin (10 µg) and it was intermediate for clindamycine (2 µg) and it has resistance for kanamycine (30 µg), amoxicillin/clavulanic acid (30 µg), ciprofloxacin (5 µg), amikacin (30 µg) and vancomycin (30 µg).

Lactobacillus plantarum proved to have resistance against kanamycine (30 µg), clindamycine (2 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), vancomycin (30 µg) and gentamycin (10 µg), and it has intermediate sensitive for erytromycin (15 µg). While, it has no resistance against the presence of streptomycine (10 µg), ampicilline (10 µg), chloramphenicol (30 µg), amoxicillin/clavulanic acid (30 µg) and rifampicin (5 µg).

Generally, such finding corresponded well with those found by Abdul-Sattar *et al.* (2011) *Lactobacilli* were found resistant to ceftazidime, ciprofloxacin, kanamycin, nalidixic acid, pipemidic acid and metronidazole. Hamilton-Miller and Shah, (1998) mentioned that among antibiotic resistances, vancomycin resistance is of major concern because vancomycin is one of the last antibiotics broadly efficacious against clinical infections caused by multi drug resistant pathogens. According to earlier reports, specific antibiotic resistance traits among probiotic strains may be desirable (Charteris *et al.,* 1998). Some authors claim that in cases of coadministration with antibiotics to prevent and treat intestinal disorders, probiotics should be resistant to certain antibiotics so as to survive in the gastrointestinal tract. However, this opinion is controversial. Probiotics containing resistance traits may have negative consequences to human health. The presence of antibiotic-resistance genes in many LAB, and the transfer of plasmids and conjugative transposons to and from LAB, have been reported in Lactobacillus species (Yoshiyuki *et al.,* 2009). The resistance to chloramphenicol, kanamycin, erythromycin, gentamycin, streptomycin and tetracycline had shown to be plasmid borne in certain probiotic cultures. Thus, there exists risk relating to potential transfer of antibiotic resistance from probiotic strains to other bacteria either commensally residing in intestine and/or pathogens (Dixit *et al.,* 2013).

Hummel *et al.* (2007) demonstrated that resistant genes might be present in probiotic strains but are silent. Genetic basis and associated resistance mechanisms towards some antibiotics are still unknown.

Table 3: Antibiotics resistance profile (µg/ml) of the three *Lactobacillus* strains in MRS after incubation at 37 ^{0}C for 24 h.

Antibiotic	Bacterial strains		
	L. acidophilus	*L. casei*	*L. plantarum*
Streptomycin 10 µg	35 (S)	29 (S)	21 (S)
Ampicilline 10 µg	39 (S)	32 (S)	24 (S)
Kanamycine 30 µg	0 (R)	0 (R)	0 (R)
Clindamycine 2 µg	16 (I)	20 (I)	13 (R)
Chloramphenicol 30 µg	31 (S)	22 (S)	20 (S)
Erytromycin 15 µg	32 (S)	35 (S)	29 (I)
Amoxicillin/Clavulanic acid 30 µg	17 (S)	13 (R)	26 (S)
Rifampicin 5 µg	22 (S)	26 (S)	21 (S)
Cefotaxime 30 µg	0 (R)	31 (S)	0 (R)
Ciprofloxacin 5 µg	0 (R)	0 (R)	15 (R)
Amikacin 30 µg	0 (R)	15 (R)	0 (R)
Vancomycin 30 µg	0 (R)	12 (R)	0 (R)
Gentamycin 10 µg	0 (R)	17 (S)	0 (R)

Measure of diameter inhibition zone (mm), (I): Intermediate, (S): Susceptibility (R): Resistance

Antibacterial activity of lactobacillus strains

Inhibition of pathogenic bacteria growth is listed one of the major desirable probiotic bacteria properties. Probiotics antagonizing pathogens through production of antimicrobial compounds such as nisin bacteriocin competing for pathogen binding and receptor sites as well as for available nutrients and growth factors (Jamaly *et al.,* 2011). In Table (4) the results of inhibition (inhibition diameter) showed that, all indicator strains are inhibited by cell-free neutralized supernatants (obtained from lactobacillus strains grown in MRS broth at 37 ^{0}C overnight) these results indicate that, Lactobacillus strains are capable of synthesizing inhibiting substance produced by tested *lactobacillus* strains differently act on pathogenic reference indicator strains. The gram-positive pathogenic bacteria could be attributed to the particular nature of their envelope, the mechanisms of action described for bacteriocins bringing in a phenomenon of adsorption (Savadog *et al.*, 2004).

Table 4: In vitro antibacterial activity of cell free supernatant of Lactobacilli against indicator organisms.

Indicator strain	Inhibitory activity of producer strain *		
	L. acidophilus	*L. casei*	*L. plantarum*
Gram positive			
Bacillus cereus	11.5	10.0	8.0
Staphylococcus aureus	12.0	15.5	9.0
Listeria monocytogenes	13.5	13.5	12.5
Gram negative			
Escherichia coli	10.5	12.5	8.0
Klebsillape numoneae	8.5	8.5	7.0
Pseudomonas aeruginosa	11.0	7.5	8.0
Salmonella typhumurium	7.0	10.0	8.5

** Measure of diameter inhibition zone (mm)*

Conclusion

From the present study it could be concluded that *Lactobacillus acidophilus* ATCC 20552, *Lactobacillus casei* DSM 20011 and *Lactobacillus plantarum* ATCC 14917 had a good probiotic characteristics in terms of acid tolerance, bile tolerance, antibiotic sensitivity and antibacterial activity against different pathogens and could be used as potential functional probiotics in food industry for commercial use.

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