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Characterization and Potential Probiotic Attributes of *Lactobacillus plantarum* DU10 Isolated from Algerian Raw Camel Milk

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Abstract: Lactic acid bacteria have long been considered safe and suitable for human consumption when administered in adequate amounts. The aim of this study is to investigate the probiotic properties of *Lactobacillus plantarum* DU10 isolated from Algerian raw camel milk. The analysis of haemolytic and gelatinase activity, bile tolerance, cholesterol lowering capacity, acid resistance, exopolysaccharide and biomolecule production were established. The results revealed normal growth of *Lactobacillus plantarum* in the presence of bile salt concentrations, gastric and intestinal fluid. This strain was able to reduce cholesterol and the majority of cells were viable at pH 2.0 even after 24 h of incubation and can produce exopolysaccharides and antimicrobial compounds. No gelatinase and haemolytic activity was detected. Natural resistance to some antibiotics was observed. According to these results, this probiotic strain could be proposed as adjunct culture and exploited for food manufacture as a biocontrol agent.

Key words: *Lactobacillus plantarum*, probiotic, lactic acid bacteria, camel milk, antimicrobial

INTRODUCTION

In Algeria, camel milk represents an important probiotic source, in which *Lactobacillus plantarum* was the major species of lactic acid bacteria isolated from this product (Zineddine *et al.*, 2011; Marroki *et al.*, 2011).

These bacteria beneficially affect the host upon ingestion by improving the balance of the intestinal microflora and are important for the maturation of the immune system, development of normal intestinal morphology and maintaining a chronic and immunological balanced inflammatory response (Tannock, 2004).

Probiotic bacteria must surmount physical and chemical barriers like acid and bile concentrations in the gastrointestinal tract to provide health profit (Liong and Shah, 2005).

The extremely acidic condition of the stomach requires that the organism should have a high tolerance to acid. This is usually determined by estimating its ability to survive to pH 3 or lower for 3 h, an average passage time before to reach the stomach. Similarly, bacterial strains need sufficient tolerance to bile to permit safe passage through the duodenum to their site of action (O'Sullivan, 2001).

Earlier studies have confirmed that some lactobacilli could decrease total cholesterol and Low-Density Lipoprotein (LDL) cholesterol (Anderson and Gilliland, 1999; Sanders *et al.*, 2000). The exact mechanism of cholesterol reduction by probiotic bacteria remains skeptical. It was also postulated that some strains of lactobacilli assimilate or incorporate some of the cholesterol removed from medium into the cellular membranes during growth (Noh *et al.*, 1997). As a result cholesterol incorporated into or adhered to the bacterial cells would be less accessible for absorption from the intestine into the blood.

Therefore, the objective of this study was to isolate and identify *Lactobacillus plantarum* from Algerian raw camel milk and evaluate its potential probiotic attributes and ability to produce antimicrobial compounds.

MATERIALS AND METHODS

Sampling: Lactic Acid Bacteria (LAB) were isolated from raw camel milk in southern Algerian Sahara, from Ouargla (Afran village, 786 km far from the capital Algiers). Milk samples were collected in sterilized bottles and bought to Applied Microbiology Laboratory at Es-Senia Oran

University (Algeria), then to the Department of Biotechnology in Pondicherry University (India) for further analysis.

Identification of lactic strains: From 127 isolates, 50 strains were retained. All isolates were Gram-positive, rod-shaped, no spore-forming and catalase-negative according to methods and criteria of Carr *et al.* (2002), Axelsson (2004) and Hammes and Hertel (2006). Growth at different temperatures (15 and 45°C) was followed in MRS broth after incubation for 24-48 h. Gas production from glucose and gluconate was determined in MRS broth containing inverted Durham and hydrolysis of arginine was tested on M16BCP medium (Thomas, 1973; Zineddine *et al.*, 2011). For the identification of *Lactobacillus* species, API 50 CHL carbohydrate fermentation strips (bioMérieux, France) were used.

Genetic identification of the selected strain: Among 50 strains of LAB, only one strain was selected and used in this study. The identification was determined by phenotypical, physiological methods and finally by 16S rDNA sequencing using universal primers 16S1 (5'AGA GTT TGA TCC TGG CTC AG 3'), 16S2 (5' ACG GCT ACC TTG TTA CGA CTT 3') and a PCR product purification kit (Sigma-Aldrich).

PCR amplification was carried out using a gradient thermal cycler (Eppendorf, Germany) programmed for 30 cycles. The reaction mix was carried out totally in 25 µL containing water (7.5 µL), *Taq* buffer (3 µL), dNTP (2.5 µL), both forward and reverse primers (2 µL), *Taq* polymerase (5 µL) and template DNA (5 µL). The cycling is preceded by a denaturation temperature at 95°C for 4 min, then a heating reaction at 95°C for 30 sec which causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules, followed by annealing temperature at 55°C for 30 sec which needs to be low enough to allow for hybridization of the primer to the strand but high enough in order for the hybridization to be specific.

Then, the extension step at 72°C for 30 sec where the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5'-3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent DNA strand. Finally, an elongation at 72°C for 10 min to ensure that any remaining single-stranded DNA is fully extended.

The PCR product was separated on a 1% agarose gel and purified using quick PCR purification spin columns (Qiagen). The purified fragment was sequenced in Microsynth Laboratories (Switzerland).

The obtained sequence was blasted in NCBI website and the results submitted to GenBank (Kumar *et al.*, 2010; Marroki *et al.*, 2011).

Antimicrobial spectrums: An overnight culture of DU10 in MRS was centrifuged for 10 min at 15 rpm. Cell Free Supernatants (CFS) were filter-sterilized through a 0.22 µm pore size Millex-GV filter (Millipore, USA) to remove residual cells.

The antimicrobial activity of CFS was determined by agar well diffusion method against both Gram positive and negative bacteria. Indicator pathogenic strains were poured separately on MRS agar petri dishes and then 4 mm wells were filled with 80 µL of CFS of *Lactobacillus plantarum* using different dilutions and fixed pH 6.5. Clear zones around each well confirmed the antimicrobial activity of the selected lactic acid bacterium (Kumar and Arul, 2009; Anas *et al.*, 2012).

Sensitivity to antibiotics: Twenty five milliliter of MRS agar was inoculated with 12 h grown strain and mixed carefully, then poured on petri dish. After solidification, different antibiotic discs were plated above the medium and incubated at 37°C for 24 h. The presence of clear zones of inhibition indicated the sensitivity of DU10 towards selected antibiotics. Results were compared to Antibiogram Committee of the French Microbiology Society (ACFMS) to confirm the obtained results. The dilution method described by Sahn and Washington (1991) was used to determine the Minimum Inhibitory Concentration (MIC) (Danielsen and Wind, 2003; Vlkova *et al.*, 2006).

Assay of gelatinase activity: Brain Heart Infusion (BHI) agar plates containing peptone (10 g L⁻¹) and gelatin (30 g L⁻¹) were used for the gelatinase production. After incubation at 37°C, cultures were placed at 4°C for 5 h before examination for liquid gelatin hydrolyze which is an indication for positive activity (Harrigan and McCance, 1990).

Assay of haemolytic activity: This analysis was determined using human blood erythrocytes (50 g 100 mL⁻¹) mixed with Brain Heart Infusion (BHI) agar (Linaje *et al.*, 2004). After inoculation and incubation, plates were examined for haemolysis which appeared as clear zones around colonies. Their nature was interpreted as β or α-haemolysis (Maragkoudakis *et al.*, 2006).

Bile tolerance: Bile tolerance was determined as described by Spencer and de Spencer (2001). The selected strain was cultivated for 16 h in MRS broth at 37°C. Harvested cells were washed twice with 0.1 M phosphate buffer, resuspended to the original volume of the same buffer and then vortexed. Only 0.5% of the suspension was used to inoculate MRS broth and MRSO supplemented by bile oxgall to obtain a final concentration of 0.05, 0.1, 0.15 and 0.3% then incubated directly at 37°C. Absorbance was read at 560 nm every hour during 24 h of incubation (Pereira and Gibson, 2002).

Cholesterol lowering capacity: MRSO supplemented with 0.3% oxgall as a bile salt was used. Water soluble cholesterol was sterilized and added to MRSO with a final concentration of 100 µg mL⁻¹, then inoculated with the selected strain and incubated for 20 h at 37°C. Cells were harvested and the remaining cholesterol concentration was determined using the method described by Rudel and Morris (1973).

Acid resistance: Artificial gastric juice (NaCl 0.2%, Pepsin 3.2 g L⁻¹, pH 2.0) was prepared and sterilized. As a control, 30 mL was adjusted to a final pH 6.0 with 1 N NaOH and then 2 mL containing approximately 8 log CFU mL⁻¹ of viable cells were added. Both media were incubated in an orbital shaker at 100 rpm at 37°C. Samples were collected at the beginning, then after 24 h and tested for cell viability using MRS agar method as described previously by Spencer and de Spencer (2001) and Pelinescu *et al.* (2009).

Exopolysaccharide production: Overnight cultures were streaked on ruthenium red milk plates (skim milk powder, 10% w/v, sucrose 1% w/v, ruthenium red 0.08 g L⁻¹ and agar-agar 1.5% w/v) and incubated at 37°C overnight. Non-ropy strains give red colonies due to the staining of the bacterial cell wall while ropy strains appear as white colonies as reported by Mora *et al.* (2002).

RESULTS AND DISCUSSION

Camel milk is one of the best sources of probiotic bacteria. Among the 50 isolates, only one was selected for its probiotic characteristics. On solid MRS medium, the selected strain gave small lenticular colonies with a milky color and a regular circumference. The microscopic examination revealed a Gram positive rod-shaped form associated in pairs or chains of varying length. These data can only guide to the genus level identification. The first observation conducted to the genus *Lactobacillus* sp.

The strain was primarily differentiated by its patterns of carbohydrate fermentation. However, when the

fermentation pattern of the isolate was compared with the identification key in Bergey’s Manual, significant differences were noted (Kandler and Weiss, 1986). The morphological, physiological and biochemical characters showed that the retained isolate was related to *Lactobacillus plantarum* species which are able to ferment sucrose, arabinose, cellobiose and xylose (Table 1).

The Genomic DNA was purified using a purification Kit (Sigma-Aldrich) according to the manufacture protocol. To check the quality of the PCR product and the genomic DNA, a gel electrophoresis was run before to send for sequencing (Fig. 1).

Table 1: Physiological and biochemical characters of *Lactobacillus plantarum*

Chemicals	Characteristics
Gram	+
Catalase	-
Arginine	+
Gaz	-
Acetones	+
Esculine	-
Galactose	-
Fructose	+
Arabinose	+
Raffinose	+
Mannose	-
Mannitol	+
Maltose	+
Xylose	+
Cellobiose	+
Sucrose	+
Ribose	+
Saccharose	+
Melibiose	+
Sorbitol	+
Glucose	+
Lactose	+

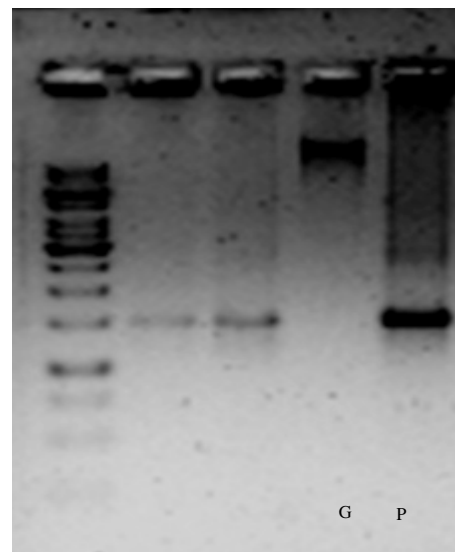


Fig. 1: Gel electrophoresis of *L. plantarum*, P: PCR product, G: Genomic DNA

The identification was further confirmed by 16S rDNA sequence analysis using GenBank and ribosomal databases (99%) and data was deposited under the accession number KF724943. The origin sequence of *Lactobacillus plantarum* DU10 is:

GCTATANTGCAGTCGACGAACTCTGGTATTGATGGTGC
 TTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGG
 TGAGTAACACGTGGGAAACCTGCCAGAAAGCGGGGAT
 AACACCTGGAAACAGATGCTAATACCCATAACAACCTTG
 GACCGCATGGTCCGAGTTTGAAGATGGCTTCGGCTATC
 ACTTTTGGATGGTCCCGCGGGTATTAGCTAGATGGTGA
 GGTAACGGCTCACCATGGCAATGATACGTAGCCGACCT
 GAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCC
 CAAATCCTACGGGAGGCAGCAGTAGGGAATCTTCCAC
 AATGGACGAAAGTTGATGGAGCAACGCGCGTGAAGTG
 AAGAAGGGTTTCGGCTCGTAAACTCTGTTGTTAAAGA
 AGAACATATCTGAGAGTAACTGTTTCAGGTATTGACGGT
 ATTTAACAGAAAGCCACGGCTAACTACGTGCCACGAG
 CCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTT
 ATTTGGGCGTAAAGCGAGCGCAGGCGGTTTTTAAAGTCT
 GATGTGAAAAGCTTCGGCTCAACCGAAGAAGTGCATCG
 GAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAA
 CTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAG
 AACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC
 TCGTGGAGGCTCGAAAGTATGGGTAGCAAACAGGATTA
 GATACCTGGTAGTCCATACCGTAAACGATGAATGCTA
 AGTGTGGAGGGTTCCGCCCTTCAAGTGTGCTGACGCTAA
 CGCATTAAAGCATTCCGCTGGGGAGTACGGCCGCAAGG
 CTGAAACTCAAAGAAATTGACGGGGGCCGACAAAGCG
 GTGGAGCATGTGGTTTAAATTCGAAGCTACGCGAAGAAC
 CTTACCAGGTCTTGACATACTATGCAAATCTAAGAGAT
 TAGACGTTCCCTTCGGGGA

CSF of *L. plantarum* DU10 gave clear zones around the indicator pathogenic strains. The largest diameter of inhibition which is 20 mm was obtained using *Listeria monocytogenes*, *Listeria innocua*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Table 2 indicates that DU10 is able to inhibit the growth of both Gram positive and negative bacteria.

DU10 was found to have a wide antimicrobial spectrum against pathogenic bacteria. In fact, *L. plantarum* have been shown *in vitro* to possess anti-microbial activity against potentially pathogenic species such as *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Yersinia enterocolitica*, *Citrobacter freundii*, *Enterobacter cloacae* and *Enterococcus faecalis* (Jacobsen *et al.*, 1999) and relatively powerful antagonistic properties against *Salmonella enterica* subsp. *enteric* and more intermediate antagonistic activity against *Helicobacter pylori* (Hutt *et al.*, 2006).

The bio-assay of antibiotic showed that DU10 was resistant to vancomycin, cefpodoxime, polymyxin and sulfaphurazole (Table 3).

Various reports indicated that *Lactobacillus plantarum* was naturally resistant to some antibiotics, such as penicillin G, ampicillin, vancomycin, chloramphenicol or ciprofloxacin.

These results are not in concordance with the present result, except for vancomycin (Halami *et al.*, 2000;

Coppola *et al.*, 2005). This antibiotic belongs to glycopeptide antibiotics and inhibits the peptidoglycan synthesis which is important structural component of bacterial cell wall. Therefore, lactic acid bacteria are especially vulnerable to vancomycin treatment (Reynolds, 1989).

Lactobacillus plantarum showed no positive haemolysin and gelatinase activity comparing to the control strain of *Staphylococcus aureus* ATCC 6538. Neither clear zones around colonies (α -haemolysis), nor green-hued zones (β -haemolysis) were observed. This criterion is considered to be important for starter use in dairy industry (Marroki and Bousmaha-Marroki, 2014).

To be a successful probiont, a bacterium must resist harsh conditions in stomach and gut regions; it must be able to colonize intestinal epithelium for its probiotic action.

Growth pattern of *L. plantarum* was normal in different concentrations of bile salt as shown by the growth curve (Fig. 2).

Normal growth was observed in different concentrations of MRSO comparing to MRS culture. It may due to the production of bile hydrolase enzyme by DU10.

Table 2: Antimicrobial activity of *Lactobacillus plantarum* DU10 toward pathogenic bacteria

Pathogenic strain	Code	Sensitivity
<i>Listeria monocytogenes</i>	ATCC 15313	+++
<i>Listeria innocua</i>	ATCC 33090	+++
<i>Staphylococcus aureus</i>	ATCC 6538	+++
<i>Pseudomonas aeruginosa</i>	ATCC 27853	+++
<i>Escherichia coli</i>	ATCC 8739	++
<i>Klebsiella pneumonia</i>	ATCC 700603	+
<i>Salmonella enterica</i>	ATCC 14028	++
<i>Salmonella typhi</i>	ATCC 700720	++
<i>Vibrio fischeri</i>	ATCC 700601	+
<i>Vibrio parahaemolyticus</i>	ATCC 17802	+

Clear zones observed less than 5 mm: +, ++: Between 5 and 10 mm, +++: More than 10 mm

Table 3: Antibiotic sensitivity of *Lactobacillus plantarum*

Antibiotics tested	Concentration ($\mu\text{g disc}^{-1}$)	Sensitivity
Chloramphenicol	30	S
Amoxycillin	10	S
Vancomycin	30	R
Gentamycin	10	S
Oxytetracycline	30	S
Erythromycin	10	S
Amikacin	30	S
Penicillin	10	S
Cefpodoxime	10	R
Neomycin	30	S
Methicillin	30	S
Novobiocin	30	S
Kanamycin	30	R
Rifampicin	30	S
Tetracycline	5	S
Ampicillin	10	S
Polymyxin	300	R
Sulfaphurazole	300	R
Ciprofloxacin	5	S

S: Sensitive, R: Resistant

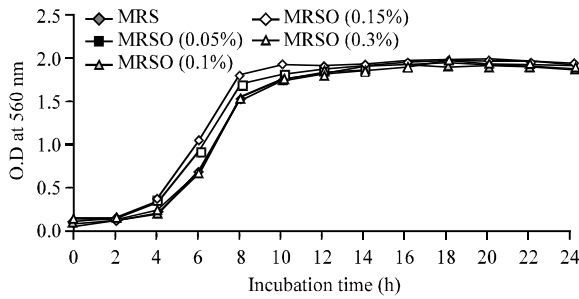


Fig. 2: Kinetic growth of *Lactobacillus plantarum* in different bile salt concentrations

Table 4: Effect of artificial gastric juice on the growth of DU10

Incubation time (h)	pH 2.0		pH 6.0	
	----- (log CFU mL ⁻¹) -----		----- (log CFU mL ⁻¹) -----	
0	6.99±0.03		8.12±0.12	
24	6.02±0.01		7.67±0.06	

In case of *L. plantarum* absorbance at 550 nm, was 0.241±0.02 for standard 100 µg mL⁻¹ cholesterol (inoculated MRSCHO broth). Absorbance of test sample was 0.246±0.03. Residual cholesterol in the inoculated MRSCHO broth (MRS broth with 0.3% bile salt and 100 µg mL⁻¹ cholesterol) was determined to be 13.79 µg mL⁻¹.

This bacterium was able to survive to artificial gastric juice up to 24 h of incubation however the bacterial count was lower compared to control (pH 6.0).

Initially, when *L. plantarum* was inoculated, its count was 6.99±0.03 log CFU mL⁻¹ at pH 2.0 and 8.12±0.12 log CFU mL⁻¹ at pH 6.0 which was reduced to 6.02±0.01 log CFU mL⁻¹ and 7.67±0.06 log CFU mL⁻¹, respectively after 24 h (Table 4).

Also as mentioned in introduction section cholesterol reduction is a special property of probiotic bacteria which is indirectly related to bile salt hydrolase activity of the bacterium (Soccol *et al.*, 2010).

All the stained colonies with Ruthenium Red of *L. plantarum* appeared to be white and non-ropy; it means that DU10 have produced exopolysaccharide according to Mora *et al.* (2002). Production of EPS prevents the staining of the bacterial cell wall and hence ropy colonies appear white on the same plates (Stingele *et al.*, 1996). Exopolysaccharides play a main industrial role for fermented products, especially for the production of yoghurt, drinking yoghurt, cheese, fermented cream and milk-based desserts (De Vuyst and Degeest, 1999).

EPS are associated with microbial cells protection against extreme environmental conditions (De Vuyst and Degeest, 1999). These biomolecules can play also an important role in the colonization of lactic acid bacteria to intestinal mucosa and by enhancing the immunity of host (Gorska *et al.*, 2010).

EPS are used as biothickeners because of their benefic properties in food industries and it has been shown that they confer immunomodulatory, antitumor, antibiofilm and antioxidant activity to consumers (Wang *et al.*, 2008) which have received intensive interest because of their safe natural source (Yang *et al.*, 2013).

Lactobacillus plantarum is a widespread Gram-positive bacterium commonly found in fermented foods and has many applications as a starter culture as a probiotic and in therapeutics delivery (Ramiah *et al.*, 2008). *Lactobacillus plantarum* occupies a wide range of niches due to its ability to utilize a broad array of carbohydrates as growth substrates and to grow and survive at low pH (Ingham *et al.*, 2008).

CONCLUSION

In summary, *L. plantarum* DU10 showed positive traits which give the isolated strain a good probiotic potential. Some additional studies should be conducted to know the power of adhesion and its stability to manufacturing processes. The *in vitro* screening of Lactobacilli from raw camel milk constitutes a valuable strategy for the large level preliminary selection of putatively safe LAB expected for use as probiotic cultures.

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REFERENCES

- Anas, M., B.A. Zinedine, H.A. Rizk, H.J. Eddine and K. Mebrouk, 2012. Screening of autochthonous *Lactobacillus* species from Algerian raw goats' milk for the production of bacteriocin-like compounds against *Staphylococcus aureus*. Afr. J. Biochem., 11: 4595-4607.
- Anderson, J.W. and S.E. Gilliland, 1999. Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolemic humans. J. Am. Coll. Nutr., 18: 43-50.

- Axelsson, L., 2004. Lactic Acid Bacteria: Classification and Physiology. In: Lactic Acid Bacteria: Microbiological and Functional Aspects, Salmien, S., A. Von Wright and A. Ouwehand (Eds.). 3rd Edn., Marcel Dekker, New York.
- Carr, F.J., D. Chill and N. Maida, 2002. The lactic acid bacteria: A literature survey. *Crit. Rev. Microbiol.*, 28: 281-370.
- Coppola, R., M. Succi, P. Tremonte, A. Reale, G. Salzano and E. Sorrentino, 2005. Antibiotic susceptibility of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. *Lait*, 85: 193-204.
- Danielsen, M. and A. Wind, 2003. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *Int. J. Food Microbiol.*, 82: 1-11.
- De Vuyst, L. and B. Degeest, 1999. Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol. Rev.*, 23: 153-177.
- Gorska, E., K. Popko, A. Stelmazczyk-Emmel, O. Ciepiela, A. Kucharska and M. Wasik, 2010. Leptin receptors. *Eur. J. Med. Res.*, 15: 50-54.
- Halami, P.M., A. Chandrashekar and K. Nand, 2000. *Lactobacillus farciminis* MD, a newer strain with potential for bacteriocin and antibiotic assay. *Lett. Applied Microbiol.*, 30: 197-202.
- Hammes, W. and C. Hertel, 2006. The genera *Lactobacillus* and *Carnobacterium*. *Prokaryotes*, 4: 320-403.
- Harrigan, W.F. and M.E. McCance, 1990. Laboratory Methods in Food and Dairy Microbiology. Academic Press, London.
- Hutt, P., J. Shchepetova, K. Loivukene, T. Kullisaar and M. Mikelsaar, 2006. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. *J. Applied Microbiol.*, 100: 1324-1332.
- Ingham, C.J., M. Beerthuyzen and J.H. Vlieg, 2008. Population heterogeneity of *Lactobacillus plantarum* WCFS1 microcolonies in response to and recovery from acid stress. *Applied Environ. Microbiol.*, 74: 7750-7758.
- Jacobsen, C.N., V.R. Nielsen, A.E. Hayford, P.L. Moller and K.F. Michaelsen *et al.*, 1999. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. *Applied Environ. Microbiol.*, 65: 4949-4956.
- Kandler, O. and N. Weiss, 1986. Regular, Non-Spore-Forming Gram Positive Rods. In: *Bergey's Manual of Systematic Bacteriology*, Sneath, P.H.A., N. Mair, M.E. Sharpe and J.G. Holt (Eds.). Vol. 2, Williams and Wilkins, Baltimore, pp: 1208-1234.
- Kumar, R.S. and V. Arul, 2009. Purification and characterization of phocaecin PI80: An anti-listerial bacteriocin produced by *Streptococcus phocae* PI80 Isolated from the gut of *Peneaus indicus* (Indian white shrimp). *J. Microbiol. Biotechnol.*, 19: 1393-1400.
- Kumar, R.S., D.R. Varman, P. Kanmani, N. Yuvaraj, K.A. Paari, V. Pattukumar and V. Arul, 2010. Isolation, characterization and identification of a potential probiont from South Indian fermented foods (*Kallappam*, *Koozh* and *Mor Kuzhambu*) and its use as biopreservative. *Prob. Antimicrob. Prot.*, 2: 145-151.
- Linaje, R., M.D. Coloma, G. Perez-Martinez and M. Zuniga, 2004. Characterization of faecal enterococci from rabbits for the selection of probiotic strains. *J. Applied Microbiol.*, 96: 761-771.
- Liong, M.T. and N.P. Shah, 2005. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *J. Dairy Sci.*, 88: 55-66.
- Maragkoudakis, P.A., G. Zoumpopoulou, C. Miaris, G. Kalantzopoulos, B. Pot and E. Tsakalidou, 2006. Probiotic potential of *Lactobacillus* strains isolated from dairy products. *Int. Dairy J.*, 16: 189-199.
- Marroki, A., M. Zuniga, M. Kihal and G. Perez-Martinez, 2011. Characterization of *Lactobacillus* from Algerian goat's milk based on phenotypic, 16S rDNA sequencing and their technological properties. *Braz. J. Microbiol.*, 42: 158-171.
- Marroki, A. and L. Bousmaha-Marroki, 2014. Lactobacilli isolated from Algerian goat's milk as adjunct culture in dairy products. *Braz. Arch. Biol. Technol.*, 57: 410-420.
- Mora, M.L., P. Cartes, R. Demanet and I.S. Cornforth, 2002. Effects of lime and gypsum on pasture growth and composition on an acid and ISOL in Chile, South America. *Commun. Soil Sci. Plant Anal.*, 33: 2069-2081.
- Noh, D.O., S.H. Kim and S.E. Gilliland, 1997. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. *J. Dairy Sci.*, 80: 3107-3113.
- O'Sullivan, D.J., 2001. Screening of intestinal microflora for effective probiotic bacteria. *Agric. Food Chem.*, 49: 1751-1760.
- Pelinescu, D.R., E. Sasaman, M.C. Chifiriuc, I. Stoica and A.M. Nohit *et al.*, 2009. Isolation and identification of some *Lactobacillus* and *Enterococcus* strains by a polyphasic taxonomical approach. *Rom. Biotechnol. Lett.*, 14: 4225-4233.
- Pereira, D.I.A. and G.R. Gibson, 2002. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. *Applied Environ. Microbiol.*, 68: 4689-4693.

- Ramiah, K., C.A. van Reenen and L.M.T. Dicks, 2008. Surface-bound proteins of *Lactobacillus plantarum* 423 that contribute to adhesion of Caco-2 cells and their role in competitive exclusion and displacement of *Clostridium sporogenes* and *Enterococcus faecalis*. Res. Microbiol., 159: 470-475.
- Reynolds, P.E., 1989. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur. J. Clin. Microbiol. Infect. Dis., 8: 943-950.
- Rudel, L.L. and M.D. Morris, 1973. Determination of cholesterol using *o*-phthalaldehyde. J. Lipid Res., 14: 364-366.
- Sahm, D.F. and J.A. Washington, 1991. Antibacterial Susceptibility Tests: Dilution Methods. In: Manual of Clinical Microbiology, Balows, A. (Ed.). 5th Edn., American Society for Microbiology, Washington, DC., USA., ISBN-13: 9781555810290, pp: 1105-1116.
- Sanders, D.J., H.J. Minter, D. Howes and P.A. Hepburn, 2000. The safety evaluation of phytosterol esters. Part 6. The comparative absorption and tissue distribution of phytosterols in the rat. Food Chem. Toxicol., 38: 485-491.
- Socol, C.R., L.P.S. Vandenberghe, M.R. Spier, A.B.P. Medeiros and C.T. Yamagishi *et al.*, 2010. The potential of probiotics: A review. Food Technol. Biotechnol., 48: 413-434.
- Spencer, J.F.T. and A.L.R. de Spencer, 2001. Food Microbiology Protocols. Vol. 14, Humana Press Inc., Totowa, New Jersey, ISBN-13: 9781592590292, Page: 495.
- Stingle, F., J.R. Neeser and B. Mollet, 1996. Identification and characterization of the *eps* (Exopolysaccharide) gene cluster from *Streptococcus thermophilus* Sfi6. J. Bacteriol., 178: 1680-1690.
- Tannock, G.W., 2004. A special fondness for lactobacilli. Applied Environ. Microbiol., 70: 3189-3194.
- Thomas, T.D., 1973. Agar medium for differentiation of *Streptococcus cremoris* from the other bacteria. N. Z. J. Dairy Sci. Technol., 8: 70-71.
- Vlkova, E., I. Trojanova and V. Rada, 2006. Distribution of *Bifidobacteria* in the gastrointestinal tract of calves. Folia Microbiologica, 51: 325-328.
- Wang, Y., Z. Ahmed, W. Feng, C. Li and S. Song, 2008. Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir. Int. J. Biol. Macromol., 43: 283-288.
- Yang, Z., J. Xu, Q. Fu, X. Fu, T. Shu, Y. Bi and B. Song, 2013. Antitumor activity of a polysaccharide from *Pleurotus eryngii* on mice bearing renal cancer. Carbohydr. Polym., 95: 615-620.
- Zineddine, B.A., M. Anas, H.A. Rizk, H.J. Eddine and K. Mebrouk, 2011. Identification and characterization of functional and technological *Lactobacillus plantarum* strains isolated from raw goat and camel milk collected in Algeria. J. Pure Applied Microbiol., 5: 553-566.