

Study of the Antimicrobial and Probiotic Effect of *Lactobacillus plantarum* (P6) Isolated from Raw Goat's Milk from the Region of Western Algeria

¹Mami Anas, ²Kerfouf Ahmed and ¹Kihal Mebrouk

¹Laboratory of Applied Microbiology, Department of Biology, Faculty of Sciences,
University of Oran, BP 16, Es-senia, 31100, Oran, Algeria.

²Laboratory of Ecology and development of species, Faculty of Science,
University Djilali Liabes, BP89, Sidi Bel Abbès, 22000, Algeria

Abstract: The evolution from a spontaneous fermentation to a directed one is realised with selected lactic starters which give many dairy products processing various organoleptic characters. The integration of new lactic acid bacteria strains isolated from diverse ecosystems is now used to increase the duration of bio-preservation of dairy products. Moreover, some lactic acid bacteria probiotic activity is exploited to produce functional food. The aim of this study is the research of the possible probiotic and technological potential with some preventive and therapeutic characteristics of some *Lactobacillus* species isolated from Algerian's raw goat's milk. The selected isolate was identified to species level as *Lactobacillus plantarum* (P6) using API 50CH Kits. Microbiological and biotechnological techniques are used to fulfill this work. Results obtained have shown that *Lactobacillus plantarum* (P6) can resist to acidic, basic and enzymatic stresses. So the former strain can be considered as a probiotic. Moreover, the inhibition activity of the *Lactobacillus plantarum* (P6) against pathogens strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25921, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Listeria ivanovii* ATCC 19119 and *Salmonella enterica*) is clearly obtained by testing these strains with the direct method. Finally, raw goat's milk can be defined as an ecosystem that promotes the development of a microflora with probiotic characters.

Key words: Goat's milk • *Lactobacillus* • Antimicrobial activity • Probiotic and technologic proprieties • Bio-preservation

INTRODUCTION

Lactic acid bacteria (LAB) comprise a wide range of genera and include a considerable number of species. These bacteria are the major component of the starters used in fermentation, especially for dairy products and some of them are also natural components of the gastrointestinal microflora. *Lactobacillus* is one of the most important genera of LAB [1]. During the last fifteen years, the *Lactobacillus* genus has evolved and contains to date more than 80 species. They are present in raw milk and dairy products such as cheeses, yoghurts and fermented milks [1]. *Lactobacilli* comprise a large and diverse group of gram positive, non-spore forming,

catalase negative rod bacteria, able to produce lactic acid as the main end-product of the fermentation of carbohydrates [2, 3]. They are considered as generally recognized as safe (GRAS) organisms and can be safely used as probiotics for medical and veterinary applications [4].

Probiotics, as defined in a FAO/WHO [5] report, are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Probiotics are beneficial bacteria in that they favorably alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection [6].

Corresponding Author: Mami Anas, Laboratory of Applied Microbiology, Department of Biology,
Faculty of Sciences, University of Oran, BP 16, Es-senia, 31100, Oran, Algeria.

Other physiological benefits of probiotics include removal of carcinogens, lowering of cholesterol, immunostimulating and allergy lowering effect, synthesis and enhancing the bioavailability of nutrients, alleviation of lactose intolerance [7]. In order to exert their beneficial effect, probiotics must survive in the gastrointestinal (GI) tract, persist in the host and prove safety for consumer. To survive in the gut, the organisms must be tolerant to low pH and bile toxicity prevalent in the upper digestive tract. Besides, quality assurance programmes associated with research, development, production and validation of the health benefits of these bacteria require their relevant characterization and identification.

Over the world, the research of novel probiotic strains is important in order to satisfy the increasing request of the market and to obtain new functional products. These new functional products must contain probiotic cultures more active and with better probiotic characteristics comparing to those already present on the market.

This work will be focused on the technological, antimicrobial and probiotic properties of *Lactobacillus plantarum* (P6) isolated from raw goat's milk.

MATERIAL AND METHODS

Isolation of Bacteria: Twelve samples of raw goat milk were collected from five farms in the western regions of Algeria. Samples were incubated at 37°C until coagulation. Coagulated samples were then activated in MRS broth [8] at 37°C for 24h in order to obtain enriched cultures. This culture was streaked on MRS agar medium and incubated [9-14] under anaerobic condition using a candle extinction jar with a moistened filter paper to provide a CO₂-enriched, water-vapor saturated atmosphere at 37°C for 48h. Single colonies picked off the plates were sub cultured in MRS broth at 37°C for 24h before microscopic examination. The cultures of rod-shaped bacteria were streaked on MRS agar medium for purification [11-13, 15, 16]. Purified strains were stored at -20°C in sterile MRS broth supplemented with 20% glycerol. Additionally, 0.05% cysteine was added to MRS to improve the specificity of this medium for isolation of *Lactobacillus* [17, 18].

Preliminary Identification of the Isolate: Identification of the isolate at genus level was carried out following the criteria of Sharpe [19] using morphological, phenotypic and biochemical methods. The cultures were examined microscopically for gram staining and catalase production [20]. In addition, all isolates were tested for growth at 10°C

for 10 days, 45°C for 48h and CO₂ production from glucose. The pathogenic bacteria (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25921, *Bacillus cereus*) come from the collection of the microbiology laboratory of the university hospital of Oran.

Sugar Fermentation Profile: The carbohydrate fermentation profile of purified isolate was determined using API 50 CH system (Biomérieux, France). Interpretation of these fermentation profiles were facilitated by systematically comparing all results obtained for the isolates studied with information from the computer-aid database Apiweb™ API 50 CH V5.1 software.

Simulated GI Tract Preparation: Synthesis gastric juices is prepared as described (Glucose 3,5g, NaCl 2,05g, KH₂PO₄ 0,6g, CaCl₂ 0,11g, KCl 0,37g, Pepsin SIGMA® 13,3g, Distilled water s.q.f 1000ml) and adjusted to pH 2 then conserved under freeze conditions [21].

Bile Salt Tolerance: The tolerance of lactobacilli to bile salts (BS) was evaluated in MRS supplemented with bile salts using a modified method described by Dora and Glenn [22]. Test lactobacilli isolate culture was grown for 6h in MRS broth at 37°C. An aliquot of 1ml of the 6h old culture was inoculated into 100 ml MRS broth with 0,5% of bile salts (SIGMA®) and adjusted to pH 8.5 [23]. The second contains 0.1% of trypsin (SIGMA®) and α-chymotrypsine (SIGMA®) and adjusted to pH 7 [24, 25].

Bacterial growth was monitored by determination of optical density at 650nm after 6 and 24h incubation period at 37°C. The percent difference between the variation of optical density (DO) of culture without bile salts ($\Delta DO_{0\% BS}$) and the variation of optical density of culture containing 0.2 or 0.5% bile salts ($\Delta DO_{0.2}$ or $\Delta DO_{0.5\% BS}$) would give an index of isolates surviving that can be expressed as follows:

$$\text{Surviving (\%)} = [(\Delta DO_{0\% BS} - \Delta DO_{0.2 \text{ or } 0.5\% BS}) / \Delta DO_{0\% BS}] \times 100$$

Classification criteria included four arbitrary level of bile salt tolerance: excellent if the isolate survived at 0.5% bile salt after 24h; very good if the isolate survived at 0.5% bile salt after 6h but not after 24h; good if the isolate survived at 0.2% bile salt after 24h but not at 0.5% bile salt; poor if the isolate did not survive in any experimental condition. An isolate survived if it demonstrated a surviving percentage equal or greater than 50%.

Inoculum Preparation: In order to obtain a rate of concentrations, fresh cultures of *Lactobacillus plantarum* (P6) are cultivated in MRS media (pH 6.5) and incubated at 37°C. Each two hours OD₆₀₀ are evaluated to obtain 0.4, 0.5, 0.6, 0.7 and 0.8 of these cultures. Bacterial count of OD₆₀₀ is obtained by following 1/100 dilutions and successive plating in MRS media pH 6.5 to so we can extrapolate bacterial concentrations on every OD₆₀₀.

Resistance to Antibiotics: The antibiotic susceptibility of selected acidotolerant and bile tolerant isolates was determined towards nine antibiotics, namely, penicillin G (6 µg/10 IU), Ampicillin (10µg), Amoxicillin (20µg), Erythromycin (15µg), Tetracycline (30µg), Pristinamycine (15µg), Rifampine (30µg), Cefazoline (30µg) and Ciprofloxacin (5µg). The Strain selection was based on their performance toward acid and bile salts Antibiotic susceptibility was determined semi- quantitatively using a modification of the agar overlay diffusion methods of the National Committee for Clinical Laboratory Standards [26]. Diameters of inhibition zones were measured and results were expressed in terms of resistance (R), intermediate susceptibility (I), and susceptibility (S), according to cut off levels proposed [26-28].

Survival Assays: Fresh cultures of 18hr are harvested and centrifuged at 7000 rpm during 1min, pellets are inoculated in 500µL of synthesis gastric juices; inoculums are incubated at 37°C during 30 min, 1h, 1h30 min and 2h [21, 24]. Following this test, inoculums are centrifuged and pellets are immediately flooded with 500µL of MRS added with 0,5% bile salts and incubated at 37°C during same times [23]. Afterwards, inoculums are once again harvested and audited with 500µL of MRS containing 0,1% of trypsin and α-chymotrypsin and incubated at 37°C during same times (30min, 1h, 1h30 and 2h) [24, 25]. The final pellets are added to 500 µL of physiological water and diluted until 10⁻⁸, plated on MRS pH 7 and at 37°C during 24h. Colony forming units (cfu) were enumerated after incubation at 37°C during 24 h [29-31].

Gelatinase Activity: Gelatinase activity of the most antibiotics sensitive isolates was investigated as described by Harrigan and McCance [32]. 2µl of a 6h old culture was spot-inoculated into nutrient gelatin agar. The plates were incubated anaerobically for 48h at 37°C after which they were flooded with saturated ammonium sulfate solution and observed for clear zones surrounding colonies (positive reaction for gelatin hydrolysis). A strain of *Staphylococcus aureus* ATCC 25923 was used as positive control.

Haemolysis Activity: Haemolysis activity of gelatinase negative isolates was investigated as described by Gerhardt *et al.* [33]. 2µl of a 6h old culture broth was spot- inoculated into sterile blood agar. The blood agar was prepared by adding 7% sheep-blood, that had been preserved in ethylenediaminetetraacetic acid (EDTA), into sterile blood agar base at 45°C. Plates were incubated anaerobically at 37°C for 48h after which they were observed for clear zones surrounding colonies (positive reaction for beta haemolysis). A strain of *S. aureus* ATCC 25923 was used as positive control.

Antimicrobial Activity: Antimicrobial activity of the selected probiotic isolates was checked by using the agar-spot test [29, 31]. Isolates were screened for production of antimicrobial against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25921, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Listeria ivanovii* ATCC 19119 and *Salmonella enterica* as the indicator microorganisms. An aliquot of 2µl of a 6h old producer isolate culture was spotted on MRS agar and plates were incubated anaerobically at 37°C for 48h to allow exhibition of antimicrobial compounds. Cell suspensions of the indicator microorganisms were prepared as follows: each 24h old culture of the indicator strain on Mueller Hinton Agar slant was suspended in sterile physiological saline solution (NaCl 0.9%) and the turbidity was compared to 0.5 Mc Farland (corresponding to 10⁸ cfu.ml⁻¹).

50µl of the cell suspension was inoculated in 5ml of Plate Count Soft Agar and overlaid on colonies of producer isolates. After incubation at 37°C for 24h, plates were checked for zones of inhibition surrounding the producer colonies. Inhibition was recorded as positive if the width of the clear zone around the colonies of the producer was 2mm or larger.

The agar well diffusion technique was also used to discriminate antimicrobial activity of the selected probiotic isolates due to organic acid production. The method of Mante *et al.* [34] was adapted. Isolates were cultured overnight before assay. Bacterial cultures were prepared into cell supernatant pH 7.0. 50µl sterilized free-cell neutralized supernatant was filled into the well against target microorganisms. After 24h of incubation time, the diameter of the inhibition zone was measured and scored. The representation of inhibition zone were not included in 6mm diameter of well. The inhibition zone larger than 2mm was scored positive.

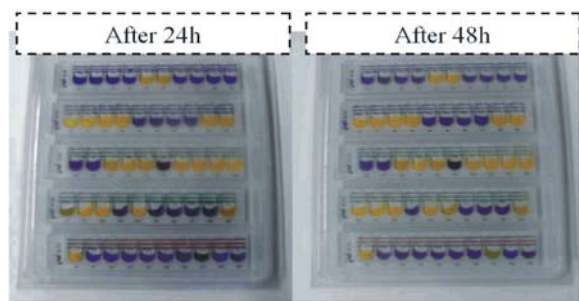


Fig. 1: Test fermentation of carbohydrates on API 50 CHL gallery of P.6 strains.

Table 1: First identification characters of lactobacilli isolates from raw goat's milk

Characters	P6
Form	rode
Gram	+
Catalase	-
Fermentation ribose	+
CO ₂ from glucose	-
CO ₂ from gluconate	+
Arginine Hydrolysis	-
Growth at 15/45	+/-
	<i>Lactobacillus</i>
Group	Group II, facultative heterofermenter (Streptobacteria)

RESULTS AND DISCUSSIONS

Choice of Effective Strain and Preliminary Identification:

This specie was chosen because of its capacity to inhibit a broad spectrum of undesirable bacteria. The identification is carried out thanks to the traditional methods established by various authors [15, 18, 35]. The latter is identified to species level by microbiological and biochemical methods, as described by Stiles *et al.* (1997). The strains retained gave small colonies of approximately 1 mm of diameter, lenticular with a white or milky color, smooth surface and a regular circular circumference were observed on solid medium. The microscopic examination revealed that the tested strains were Gram positive, with a cellular rod form associated in pairs or in chains (Table 1).

Phenotypic Identification: One isolates selected according to their good probiotic potential were identified at phenotypic level as *Lactobacillus plantarum* (P6)

using API 50 CHL technique. This isolate was from samples of various origins. Furthermore, a genotypic method was essential to discriminate between strains. The establishment of the percentage of reliability of this strain in comparison with references *Lactobacillus plantarum* ATCC 14917 (rephrase) [15, 35].

Microbiological characteristics and fermentation profile of the strain *Lb. plantarum* (P6) and of the reference strain *Lb. plantarum* ATCC 14917 are 100% identical, This similarity coefficient is calculated between P6 strain and the reference strain, What guides the identification of the strain (P6) to the species *Lactobacillus plantarum*.

Bile Salt Tolerance: After exposure to acidic conditions, one selected acidotolerant lactobacilli isolates was assayed for bile salt tolerance (Table 2). This isolate demonstrated good capacity to resist bile salts by presenting surviving percentage greater than 50% under exposure to 0.2% bile salts after 24h at 37°C. This isolate was further investigated for their safety properties including sensitivity to antibiotic, haemolysis and gelatinase activity.

Resistance to Antibiotics: One potentially probiotic lactobacilli isolates were subjected to antibiotic susceptibility testing using the agar diffusion method (Table 3). All of them were sensitive to Penicillin, Ampicillin, Amoxicilline, Tetracycline, Pristinamycine, Erythromycin and Rifampine. The isolate *Lb. plantarum* (P6) demonstrated intermediate resistance to cotrimoxazole. Notable observation is the resistance towards ciprofloxacin expressed by this isolate.

Probiotic Potential's Evaluation: The results obtained for the aptitude of the strain *Lb. plantarum* (P6) to resist acidic, basic and enzymatic stresses show clearly that this specie can be regarded as probiotic micro-organism [36]. The concentration at the end of the treatments of *Lb. plantarum* (P6) is 8.5 log ufc/ml, knowing that according to Salminen *et al.*, [36], the concentration of administration of a probiotic micro-organism must be 10⁷ or 10⁸ and that according to the definition of FAO/WHO, 2002 [5] probiotic must be administrated with a well defined concentration so that it can achieve these beneficial

Table 2: Surviving percentage of lactobacilli isolates in MRS broth supplemented with 0.2% or 0.5% bile salts after 6h and 24h at 37°C

Isolates	0,2%		0,5%	
	6h	24h	6h	24h
<i>Lactobacillus plantarum</i> (P6)	90.60	94.55	54.37	88.31
<i>Lactobacillus plantarum</i> (ATCC 14917)	85.31	88.09	51.74	85.43

Table 3: Susceptibility of potentially probiotic lactobacilli isolates to antibiotics using the disc diffusion method

Isolates	Diameter of inhibition zone in mm								
	P ₁₀	Ap ₁₀	Am ₁₀	T ₃₀	PT ₁₅	E ₁₅	RA ₃₀	C ₅	CZ ₃₀
<i>Lb. plantarum</i> (P6)	34 _(S)	31 _(S)	28 _(S)	22 _(S)	23 _(S)	27 _(S)	25 _(S)	0 _(R)	13 _(I)
<i>Lactobacillus plantarum</i> (ATCC 14917)	28 _(S)	33 _(S)	29 _(S)	21 _(S)	20 _(S)	26 _(S)	22 _(S)	0 _(R)	10 _(I)

Antibiotics (Disk potency): P₁₀: Penicillin G (10units); Ap₁₀: Ampicillin (10µg); Am₁₀: Amoxicilline (10µg); T₃₀: Tetracycline (30µg); PT₁₅: Pristinamycine (15µg); E₁₅: Erythromycin (15µg); RA₃₀: Rifampine (30µg); C₅: Ciprofloxacin (5µg); CZ₃₀: Cefazoline (30µg). (S): sensitive; (R): resistant; (I): intermediate

Table 4: Susceptibility of potentially probiotic lactobacilli isolates to antibiotics using the disc diffusion method

	OD ₆₀₀	Time			
		30mn	1h00	1h30	2h00
		Growt (Log ufc/ml)	Growt (Log ufc/ml)	Growt (Log ufc/ml)	Growt (Log ufc/ml)
<i>Lb. plantarum</i> (P6)	0,4	8,0	8	7,95	7,88
	0,5	8,1	8,14	8,11	8,07
	0,6	8,3	8,27	8,25	8,2
	0,7	8,43	8,39	8,38	8,36
	0,8	8,5	8,47	8,46	8,44

Table 5: Inhibitory activity of potentially probiotic lactobacilli isolates

Indicator strains	<i>Lb. Plantarum</i> (6)	<i>Lb. Plantarum</i> (6) (mm)
<i>Staphylococcus aureus</i> ATCC 25923	+	22
<i>Escherichia coli</i> ATCC 25922	+	14
<i>Bacillus cereus</i>	+	13
<i>Pseudomonas aeruginosa</i>	+	13
<i>Vibrio cholerae</i>	+	11
<i>Listeria ivanovii</i> ATCC 19119	+	07
<i>Salmonella enterica</i>	+	03

+: Diameter of inhibition zone = 2mm; -: No inhibition; MDR: Multi Drug Resistant.

effects for its host. In general, a probiotic micro-organism is never administrated in the form of bacterial cream, it is added as an adjuvant in agroalimentary products (Juice, yoghurt... etc) [4, 37] or in the form of capsules containing these freeze-dried micro-organisms. These methods allow, even if the species considered as probiotic resists the gastro-intestinal environment, to increase their chances to arrive in sufficient quantity at the level of intestine [38].

Haemolysis and Gelatinase Activity: The potentially probiotic *Lactobacillus* isolates was assayed for gelatinase activity and are hemolysis. It showed no activity of gelatinase and positive hemolysis compared to the positive control *Staphylococcus aureus* ATCC 25923 strains.

Antimicrobial Activity: Results for antimicrobial activity of *Lb. plantarum* (P6) probiotic was as shown in Table 5. Isolate inhibited the growth of all pathogenic strains when agar spot method was used. It was also noticed that, the neutralized free-cell supernatant from the culture of the *Lb. plantarum* (P6) inhibited the growth of all pathogenic indicators.

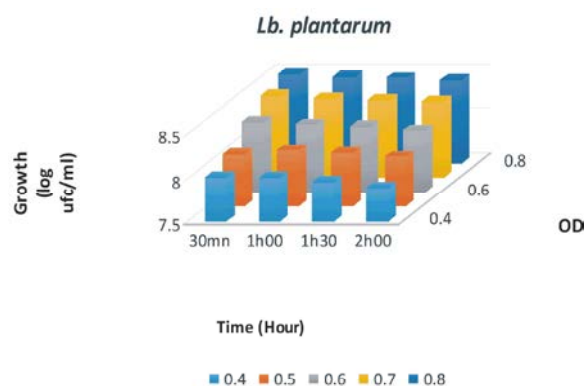


Fig. 2: Variation of concentration of *Lb. plantarum* (P6) after every treatment

Bile salt hydrolase (BSH) Activity: *Lactobacillus plantarum* (P6) inhibit the growth of pathogens, displayed BSH activity by providing dosage. *Lb. plantarum* (ATCC 14917) has an activity of average diameter of BSH demonstrating the precipitation zone of 10 mm and *Lb. plantarum* (P6) shows a strong activity of BSH by expressing the area of diameter greater than 15mm. the zone precipitation around the colonies on the plate

CONCLUSION

As a result of research work carried out at the Laboratory of Applied Microbiology, University of Oran, it could be noticed that the strain of *Lactobacillus plantarum* (P6) can be considered as a new probiotic strain potentially safer with antimicrobial properties. Then will the primer used in the future genotypic study in the agro-food industry and the manufacture of fermented dairy product.

REFERENCES

1. Coeuret, V., S. Dubernet, M. Bernardeau, M. Gueguen and J.P. Vernoux, 2003. Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. *Lait*, 83: 269-306.
2. Pelinescu, D.R., E. Sasarman, M.C. Chifiriuc, I. Stoica, A.M. Nohita, I. Avram, F. Serbancea and T.V. Dimov, 2009. Isolation and identification of some *Lactobacillus* and *Enterococcus* strains by a polyphasic taxonomical approach. *Romanian Biotechnology Letter*, 14: 4225-4233.
3. Malek, F., B. Moussa Boudjemaa, A. Aouar-Métriland and M. Kihal, 2013. Identification and genetic diversity of *Bacillus cereus* strains isolated from a pasteurized milk processing line in Algeria. *Dairy Science Technology*, 93(1): 73-82.
4. Fuller, R., 1989. Probiotics in man and animals. *Review Journal Applied of Bacteriology*, 66: 365-378.
5. FAO/WHO. 2002. Guidelines for the Evaluation of Probiotics in Food, Report of a joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, London Ontario, Canada.
6. Helland, M.H., T. Wicklund and J.A. Narvhus 2004. Growth and metabolism of selected strains of probiotic bacteria in maize porridge with added malted barley. *International Journal Food Microbiology*, 91: 305-313.
7. Parvez, S., K.A. Malik, S. Ah Kang and H.Y. Kim, 2006. Probiotics and their fermented food products are beneficial for health. *Journal Applied Microbiology*, 100: 1171-1185.
8. De Man, J.C., M. Rogosa and M.E. Sharpe 1960, Medium of Lactobacilli. *Journal Applied Bacteriology*, 23: 130-135.
9. Badis, A., 2004. Identification et caractérisation technologique de bactéries lactiques isolée à partir de lait crue de chèvre de quatre populations caprines locales. Thèse de doctorat d'état, université d'Oran.
10. Badis, A., N.R. Laouabdia-Sellami, D. Guetarni, M. Kihal and R. Ouzrout, 2006. Phenotypical characterization of the lactic bacteria isolated starting from raw goat milk from caprine local "Arabia and Kabyle". *Sciences Technology*, 23: 30-37.
11. Moulay, M., H. Aggad, Z. Benmechernene, B. Guessas, D.E. Henni and M. Kihal, 2006. Cultivable Lactic Acid Bacteria Isolated from Algerian Raw Goat's Milk and Their Proteolytic Activity. *World Journal Dairy Food Science*, 1: 12-18.
12. Moulay, M., K. Benlahcen, H. Aggad and M. Kihal, 2013. Diversity and technological properties of Predominant lactic acid bacteria isolated from Algerian raw goat's milk. *Advances in Environmental Biology*, 7(6): 999-1007.
13. Bendimerad, N., M. Kihal and F. Berthier, 2012. Isolation, identification and technological characterization of wild *Leuconostocs* and lactococci for traditional Raib type milk fermentation. *Dairy Sciences of Technology*, 92(3): 249-264.
14. Zarour, K., Z. Benmechernene, M. Hadadji, B. Moussa-Boudjemaa, J.E. Henni and M. Kihal, 2013. Microbiologique et technologique des espèces de *Leuconostoc mesenteroides* du lait cru de chèvre et de chamelle d'Algérie. *Revue «Nature and Technologie»*. B-Sciences Agronomiques et Biologiques, 08: 39-47.
15. Carr, F.J., D. Chill and N. Maida, 2002. The lactic acid bacteria: a literature survey. *Critique Review of microbiology*, 28: 281-370.
16. Benmechernene, Z., I. Fernandez., M. Kihal, K. Böhme., P. Calo-Mata and B. Velazquez, 2013. Recent Patents on Bacteriocins: Food and Biomedical Applications. *Recent Patents on DNA and Gene Sequences*, 7: 66-73.
17. Hartemink, R., V.R. Domenech and F.M. Rombouts, 1997. LAMVAB-A new selective medium for the isolation of lactobacilli from faeces. *Journal of Microbiology Methods*, 29: 77-84.
18. Kihal, M., H. Prevost, D.E. Henni, Z. Benmechernene and C. Diviès, 2009. Carbon dioxide production by *Leuconostoc mesenteroides* grown in single and mixed culture with *Lactococcus lactis* in skim milk. *Science Research and Essay*, 4(11): 1348-1353.
19. Sharpe, M.E., 1979. Identification of lactic acid bacteria, In: Skinner F.A., Lovelock D.W., (Ed), identification methods for microbiologists. Academic Press, London, pp: 233-259.
20. Harrigan, W.F. and M.E. Mc Cance, 1976. Laboratory Methods. *International Food Dairy Microbiology*. Eds Harrigan WF, Mc Cance ME. Academic press. New York. USA, pp: 12-15.

21. Fernandez, M.F., S. Boris and C. Barbes 2002. Probiotic properties of human lactobacilli strains to be in the gastrointestinal tract. *Journal Applied of Microbiology*, 94: 449-455.
22. Dora, I.A.P. and R.G. Glenn, 2002, Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. *Applied Environment Microbiology*, 68: 4689-4693.
23. Shima, M., T. Matsuo, M. Yamashita and S. Adachi 2009. Protection of *Lactobacillus acidophilus* from bile salts in a model intestinal juice by incorporation into the inner-water phase of a W/O/W emulsion. *Food Hydrocolloids*, 23: 281-285.
24. Kim, P.I., M.Y. Jung, Y.H. Chang, S. Kim, S.J. Kim and Y.H. Park, 2006. Probiotic properties of *Lactobacillus* and *Bifidobacterium* strains isolated from porcine gastrointestinal tract. *Applied Microbiology and Biotechnology*, 74: 1103-1111.
25. Ruiz-Moyano, S., A. Martin, M.J. Benito, F.P. Nevado and M. de Guia Cordoba, 2008. Screening of lactic acid bacteria and bifidobacteria for potential probiotic use in Iberian dry fermented sausages. *Meat Science*, 03: 1-11.
26. NCCLS (National Committee for Clinical and Laboratory Standard) 2002. Performance standards for antimicrobial susceptibility testing, Twelfth Informational Supplement. M100-S12.
27. Prescott, M.L., P.J. Harley and A.D. Klein, 1999. Antimicrobial chemotherapy. In *Microbiology Fourth Edition*, ISBN.USA, pp: 678-695.
28. Vlkova', E., V. Rada, P. Popela'rjova', I. Trojanova' and J. Killer, 2006. Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. *Livestock Science*, 105: 253- 259.
29. Mami, A., J.E. Henni and M. Kihal, 2008. Antimicrobial activity of *Lactobacillus* species isolated from Algerian Raw Goat's milk against *Staphylococcus aureus*. *World Journal Dairy Food Science*, 3: 39-49.
30. Boumehira, A.Z., A. Mami, J.E. Henni and M. Kihal, 2011. Identification and Characterization of Functional and Technological *Lactobacillus plantarum* Strains Isolated from Raw Goat and Camel Milk Collected in Algeria. *Journal of Pure Applied Microbiology*, 5(2): 553-566.
31. Mami, A., A.Z. Boumehira, A.R. Hamedi, J.E. Henni and M. Kihal, 2012. Screening of autochthonous *Lactobacillus* species from Algerian raw goats' milk for the production of bacteriocin-like compounds against *Staphylococcus aureus*. *African Journal of Microbiology Research*, 6(12): 2888-2898.
32. Harrigan, W.F. and M.E. Mc Cance, 1990. *Laboratory Methods in Food and Dairy Microbiology*. Academic Press, London.
33. Gerhardt, P., R.G.E. Murray, R.N. Costilow, E.W Nester, W.A Wood, N.R. Krieg and G.B. Phillips, 1981. *Manual of methos for general bacteriology*. American Society of Microbiology, Washington, DC 20006.
34. Mante, E.S., E. Sakyi-Dawson and W.K. Amoa-Awua, 2003. Antimicrobial interactions of microbial species involved in the fermentation of cassava dough into agbelima with particular reference to the inhibitory effect of lactic acid bacteria on enteric pathogens. *International Journal of Food Microbiology*, 89: 41-50.
35. Klein, G., A. Pack, C. Bonaparte and G. Reuter, 1998. Taxonomy and physiology of probiotic lactic acid bacteria. *International Journal of Food Microbiology*, 41: 103-125.
36. Perea Velez, M., K. Hermans, T.L.A. Verhoeven, S.E. Lebeer, J. Vanderleyden and S.C.J. De Keersmaecker, 2006. Identification and characterization of starter lactic acid bacteria and probiotics from Columbian dairy products. *Journal Applied Microbiology*, pp: 1-9.
37. Champagne, C.P. and N.J. Gardner, 2008. Effect of storage in a fruit drink on subsequent survival of probiotic lactobacilli to gastro-intestinal stresses. *Food Research International*, pp: 1-5.
38. Pan, X., F. Chen, T. Wu., H. Tang and Z. Zhao, 2009. The acid, bile tolerance and antimicrobial property of *Lactobacillus acidophilus* NIT. *Food Control*, 20: 598-602.