International Journal of Applied and Natural Sciences (IJANS)
ISSN (P): 2319-4014; ISSN(E): 2319-4022
Vol. 5, Issue 5, Aug – Sep 2016; 53-62

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

ENUMERATION, ISOLATION AND IDENTIFICATION PHENOTYPIC THERMOPHILE LACTIC ACID BACTERIA ISOLATED FROM DIFFERENT FERMENTED MILK COLLECTED IN ALGERIA

LOUMANI AKIL[1], BELARBI FATIMA[2] & BENSOLTANE AHMED[3]

[1]Laboratory of Drying, Unity Renewable Energy in Sahara medium Adrar, CDER, Algeria

[1,2,3]Laboratory of Biotoxicology Experimental, Biodepollution and Phytoremediation, University of Oran1 Ahmed Benbella Oran, Algeria

ABSTRACT

The lactic acid bacteria which are considered the most useful microorganism to society are involved in many manufacture fermented foods such as yogurt and cheese. The purpose of this study was to isolate and characterize the thermophile lactic acid bacteria from different fermented milk collated in Algeria. Twenty samples of different milk used for this experiment were obtained from camel in Adrar province and goat, sheep, cow in Relizane area in Algeria. A total of 50 colonies were grown in MRS acetic and LM17 agar. The pre-identification tests were performed according to the morphological characteristics such as catalase, the Gram, growth at 10C°, growth in presence 6.5%NaCl. The isolates were Subjected to different screening test and identified as presumptive lactic acid bacteria and classified to the genera *Lactobacillus* (17), *Streptococcus* (04), *Enterococcus* (29). The isolated species lactic acid bacteria using API50CHL, API20 Strep were *Lactobacillus delbrueckii bulgaricus* (10%), *Streptococcus thermophilus* (8%) and *Enterococcus faecium* (34%) *Enterococcus faecalis* (24%). Among of the lactic acid bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* produced. The high acid compared with other lactic acid bacteria. The strains showed properties suggest that they are good candidate for dairy fermentation process.

KEYWORDS: Algeria, Fermented milk, Lactic Acid Bacteria, Proprieties Technologic

INTRODUCTION

Lactic acid bacteria (LAB) are indigenes habitants in different daily product. It has been long consumed by people in several fermented food, and widely used for fermentation and preservation of a wide range of milk, meat and vegetable foods**,** (Zhu, Liu, & Wu, 2000)**.** The mechanism of fermentation the dairy products is metabolize the lactose to lactic acid, which allow to lowers the PH and creates an environment unfavorable to pathogens and spoilage organisms**.** (Aslim, Yuksekdag, Sarikaya, & Beyatli, 2005)**.***Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the most important in genera of lactic acid bacteria it's involved to many dairy product such as cheese, yoghurt. Milk is a complete food, containing proteins, fats, carbohydrates, vitamins and mineral salts (Cheriguene, Chougrani, Bekada, El Soda, & Bensoltane, 2007), it's widely used for home consumption worldwide and to produce different cheeses and yoghurts (Seifu, Abraham, Kurtu, & Yilma, 2012)**.**The objectives of this study were to collect a variety of samples milk in tow Region Relizane and Adrar located in Algeria in order to determinate the thermophile species and their technological characters.

MATERIAL AND METHOD

Microbiological Analysis

Samples

A total of 20 samples of different row milk (sheep, cow, camel and goat) were collected from two Regions, Relizane and Adrar located in Algeria. The samples were immediately cools and transported in icebox with 4 degree C° and analyzed of content of laboratory on the arrival

Table 1: Media and Condition for Enumeration and Isolation of LAB

Microorganism	Media	T(C°)	Duration	Condition
Thermophilic Lactobacilli	MRS acetic(De Man et al., 1960) addition acetic acid	42	24-36	Anaerobiosis
Streptococcus or *Enterococcus*	LM17(Terzaghi & Sandine, 1975)Addition 5g of lactose.	42	24-72	Aerobiosis

Enumeration and Isolation the Thermophile Species

Ten (10ml) of each samples of raw milk homogenized with 90 ml of saline water in order to make initial dilution (10^{-1}). The suspension was used for making suitable serial dilution up to (10^{-8}) by incorporating 1ml in 9ml of sterile saline water in sterile tubes. The enumeration was determined by using two selective media such as MRS for the bacilli and M17 for cocci agar as indicate de Table 1.After incubation, colonies were enumerated, recorded as colony forming units (UFC) per liter of milk. The colonies were randomly picked and transferred in 10ml appropriate broth. The purity of isolates was checked by streaking again and subculturing in fresh agar followed by macroscopic and microscopic examinations. The strains displaying characteristic lactic acid bacteria and considered as presumptive *Lactobacillus sp* in MRS and as presumptive *Streptococcus* or *Enterococcus* sp in M17 were chosen in order to use in further studies. The conservation for long time of isolates purified, without appreciable loss of properties was carried out to the medium containing(70%) skim milk (enriched by 0.05% of yeast extract and 0.05% of glucose) and(30%) glycerol, the isolates were stored at -80C°.the working cultures were also kept in MRS or M17 Agar slant at 4C°, renewed every 4weeks. (Hassaïne, Zadi-Karam, & Karam, 2008)**.**According to many methods of characterization of isolates recommended by several authors such as (Khedid, Faid, Mokhtari, Soulaymani, & Zinedine, 2009)**.** The isolates were Gram stained, and tested of catalase production. The Preliminary isolation and grouping on was basis of morphological and phenotypic properties using gas production from glucose, determined in M17 or MRS broth containing inverted Durham; growth at different temperature (10),(15) and (45C°) and PH 9,6 as well as the ability to grow in different concentration such as (2%),(4%) and(6.5%) w/v the Sherman test and survival after heating at 60C° for 30min (Bettache & Mebrouk, 2004)**,**hydrolysis of Arginine tested on MRS or M17 broth with bromocresol purple (Hassaïne et al., 2008) and production of acetone from glucose, determined by using the Voges-Prokauer test (Hassaïne et al., 2008)**.**

Identification of Lactic Acid Bacteria to the Species Level

The fermentation of carbohydrate was determined according to the method described by (Schillinger & Lucke, 1987) in MRS broth (without sugars) containing 1% of solution carbohydrate testing and added 0.025% bromocrsol purple as PH indicator. The carbohydrate tested were Cellobiose, Galactose, Mannitol, Melizitose, Melibiose, Ribose, Trehalose, Xylose, and Glucose, Lactose, Saccharose, Fructose and Arabinose. To ensure to anaerobic conditions, each tubes was topped up with two drops of sterile liquid paraffin after Incubation (Samelis, Maurogenakis, & Metaxopoulos, 1994)**.** The

results was recorded after 48h of incubation at 42C°. The fermentation patterns of carbohydrates were determined using the API20 strep and API50 CHL gallery with API50CHL medium (bio merieux, Marcy-l'Etoil. France). The pib win database was used to interpret the results.

Properties Technologic

Acidification Activity of Isolates

The production of acid lactic by our isolates lactic acid bacteria species was determined after growing the isolates in MRS acetic (rods) and LM17 (cocci), and then inoculating in sterile reconstituted skim milk supplemented yeast extract (3g/l) and glucose (2g/l).sterile reconstituted skim milk was inoculated with 1% of overnight culture according to method descripted by (Attia, Kherouatou, & Dhouib, 2001)**.**The inoculated culture was incubated at 42° for two type thermopiles isolates (Seifu et al., 2012) change PH was mentioned at different intervals by tacking samples at 0h (initial),(6h),(12h),(24h),(72h) until the PH reached 4.6 (iso-electric point),as suggested by (Burns et al., 2008)**.**the isolates of lactic acid bacteria species were considered as fast acid producers where the less than 12h to reach PH4.6, medium acid producers (12h,48h to reach 4.6) slow acid producers (more than 48h to reach PH4.6).

Evaluation of proteolytic activity

To evaluate proteolytic activity, The milk agar was prepared by adding 1% skim milk powder to Plate Count Agar (Beerens & Francois, 1990). The surface dried milk agar was streaked by culture overnight. The plates were incubated in 45C° in 48h.the transparent forming halo colonies were considered positive.

Statistical Analyses

Difference in acid production potential between the isolated lactic acid bacteria was determined by the analysis of variance technique using SPSS software (version 10) and Duncan Multiple Range test was used for mean separation when ANOVA showed statistical difference between means. Statistical differences were declared at 5% ($P< 0.05$) significance level (Steel, Torrie, & DICKEY, 1980)**.**

Antibacterial Activity

The inhibitory effect of stains LAB isolated and identified such as (LB_1, LB_2, LB_3) and (ST_1, E_1, E_2). Over strains pathogens was tested using two method direct and indirect: the first was the spot agar test (direct method) as carried by (Tagg, Read, & McGiven, 1971) and the second was the agar well diffusion assay as carried by (Ramadan, El-Sawi, Abdel-Glel, & Mohamed, n.d.) Using indicators bacteria pathogen such as *E. coli (ATCC 25955*, *Listeria monocytogenes (ATCC 7659) Staphylococcus aureus (ATCC 25925)*, were grown in bouillon nutritive (BN). For the direct method our strains were spotted in MRS Agar for the strains (LB_1, LB_2, LB_3) and M17 Agar for the strains (ST_1, E_1, E_2) and incubated at 42C° for 18 h.100µl of each culture indicator bacteria precedent were transferred in10ml MH than poured over the spot and re-incubated at 42C°. The results were targeted by examination the zones of inhibition. For the indirect method few colonies (4-5) were picked from each pathogen bacteria and suspended in 4ml of sterile water and standardized to approximately108CFU/ml in order to adjust standard turbidity to 0.5 of McFarland. A sterile swab were soaked in the suspension and inoculated in MHA (Muller Hinton Agar) plate. After the inoculum was added and allowed to absorb, and (6 mm) sterile paper filter discs (Whatman N°:1) moistened with 20 ml of cell free supernatant obtained by centrifugation (2500 _ g/10 min) from each isolate of LAB in exponential growth phase were added. The susceptibility of pathogens to

the discs was assessed by measuring the zone of inhibition of bacterial growth around the discs (radius - mm) after incubation for 24 h at 37 _C. A clear zone of inhibition of at least 2 mm radius was recorded as positive. The experiment was performed in triplicate.

RESULTS AND DISCUSSIONS

Enumeration of Lactic Acid Bacteria

Table 2 summarizes the microbial count obtained from various samples The LAB was enumerated using selective media such as MRS and M17 from various samples. the strains presumptive the *Streptococcus sp* or *Enterococcus sp* counts from $1x10^3$ cfu/ml for cow milk and counts ($23x10^3$ cfu/ml) for sheep milk and counts ($1.06x10^4$ cfu/ml) for goat milk and finally, counts $7.2x10^3$cfu/ml for camel milk. For the presumptive *Lactobacillus sp* counts ($39.5x10^3$ cfu/ml) for row milk and ($17.2x10^3$ cfu/ml) for sheep milk, and counts $7.8x10^3$ cfu/ml for goat milk, and counts 98.2x103 cfu/ml for camel milk . we look that the *Streptococcus sp* or *Enterococcus sp* incubated at 42C° are less than *Lactobacillus sp* at 42 C°, and also, The LAB counts to the camel milk is higher than other milk such as sheep milk (Badis et al., 2004).

Table 2: Microbial Count from Various Samples in Cfu/Ml

Strains presumptive	Goat milk	Sheep milk	Cow Milk	Camel milk
Streptococcus sp or *Enterococcus sp*	$1.06x10^4$	$2.3x10^3$	$1x10^3$	$7.2x10^3$
Lactobacillus Thremophilic	$7.8x10^3$	$1.06x10^4$	$39.5x10^3$	$7.2x10^3$

Identification of LAB

In the present study, a total of 50 isolates of the thermophilic LAB were obtained from different milk collected in Region of Algeria. These isolates were identified to genus species level based on their cellular morphology, gas production, growth at 10 C° and 45 C°, in presence of (6.5%) NaCl and in PH 9.6 according to (Holzapfel & Wood, 1995) and the biochemical test using API 20 strep and API 50 CHL. All the isolates are Gram positive, catalase negative and non-mobility and non-spores forming and thermophilic. (Seifu et al., 2012). The first screening revealed that the isolated were subdivided into 2 groups : The first contains 33 strains (14 isolates from ewe's milk, 9 isolates from camel milk, 2 isolates from sheep milk and 8 isolates from goat milk),these strains were white, round or lenticular colonies cocci, diplococci and in chine cells and homofermentative. Among to the isolates cocci, 23 isolates represented with two species, cocci were able to grow at 10 C° and in (6.5%) of NaCl and in PH 9.6 broth, and also survive at 60 C° for 30 min, capable to hydrolyze Arginine, able to grow the Sharman milk of 1%, hydrolyze of starch. In theses 23 strains , 12 isolated capable to ferment Starch, Glucose, Lactose, Maltose, Mannitol, Mannose, Melibiose, Ribose, Sucrose ,Sorbitol, Trehalose and variable for galactose and incapable to ferment Amygdaline, Arabinose, Cellobiose, Fructose, Raffinose, Rhamnose, Xylose were characterized as *Enterococcus faecalis* and 07 isolated able to form, Glucose, Lactose, Galactose, Maltose, Sucrose, Arabinose, Maltose, Mannitol, Trehalose, Cellobiose and didn't ferment Sorbitol, Raffinose, Xylose, Melibiose, Rhamnose, were characterized as *Enterococcus faecium*. The last 04 isolated were unable to grow at 10 C°, at PH 9.6.in addition, incapable to hydrolyze starch and hydrolyze esculin and Sharman test(1%) they did not survive in 60 C° for 30 min. incapable to grew at(6.5%) and at (4%). for they formed acid from Glucose, Saccharose, Lactose and Galactose and variable for Raffinose and unable to ferment maltose, Mannitol, Trehalose, Cellobiose, Melibiose, Raffinose, Xylose, Sucrose, Arabinose, Melezitose, Rhamnose, Mannitol were characterized as *Streptococcus thermophilus* (Schillinger & Lucke, 1987) . The second group comprise: 17 isolated and that were classified into 3 species. These isolates characterized as gram positive, catalase negative, rods and grew at 45 C°, these strains suggest classified as thermopiles

facultative homofermentative *Lactobacillus*. Among these isolates, 05 isolated considered as *Lactobacillus delbrueckii subsp.bulgaricus* belong to their inability to hydrolyze Arginine and esculin and also incapable to grow in (6.5 %),(2%), (4%) of NaCl and also able to form acid from fructose, glucose, lactose but they didn't fermented Arabinose, Cellobiose, Galactose, Raff nose, Xylose. 03 isolated were able to grow at (2%) of NaCl, variable to hydrolyze Argentine and able to form acid from sugars Galaxies, Fructose, Glucose and unable to Ferment sugars such as starch, Arab nose, Cellobiose, Mannose, Melibiose, xylose were characterize as *Lactobacillus delbrueckii subsp .lactis*.02 isolated Capable to from acid from the sugars Fructose, Glucose, Lactose, Maltose, Mannose, Sucrose. These isolates didn't fermented starch, Arabinose, Amygdaline, Cellobiose, Galactose, Mannitol, and were characterized as *Lactobacillus delbrueckii subsp. delbrueckii.* The last 07 isolated considered as *Lactobacillus casei subsp.rhamnosus* were characterized by fermentation the special sugar rhamnose and also the Majority of other sugars were fermented. The following table number 3 describes all characteristics physiological and biochemical of isolated.

Table3: Physiological and Biochemical characteristics of LAB Isolated from Various Fermented Milk

Identified as	**Thermophilic Rods**				**Thermophilic Cocci**		
	Lb. delbrueckii subspbulgaricus ***N=05***	***Lb. delbrueckii subsp. lactis*** ***N=03***	***Lb. delbrueckii subsp. delbrueckii .*** ***N=02***	***Lb. Casei subsp Rhamnosus*** ***N=07***	***Enterococcus faceium*** ***N=17***	***Enterococcus faecalis*** ***N=12***	***Streptococcus thermophilus*** ***N=04***
Gram	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-
Morphology	**Rod**	**Rod**	***Rod***	***Rod***	***Cocci***	***Cocci***	***Cocci***
Production of CO2 from Glucose	-	+	-	-	-	-	-
Growth at PH 9.6	-	+	-	-	-	-	-
Growth at 10C°	-	-	-	-	-	-	-
45C°	+	-	-	-	-	-	-
60C°from 30 Min	+	-	-	-	-	-	-
NaCl	-	+	-	-	-	-	-
2%	-	+	-	-	-	-	-
4%	-	-	-	-	-	-	-
6.5%	-	-	-	-	-	-	-
Sharman test (1%)	-	-	-	-	-	-	-
Hydrolyse of Arginine (ADH)	-	-	-	-	-	-	-
Acetoin (VP)	-	-	-	-	-	-	-
Hydrolyse starch	-	-	-	-	-	-	-
Hydrolyse Esculin	-	-	_	_	-	_	-
Acid From Starch	_	_	_	_	_	-	-
Amygdalin	-	-	-	-	-	-	-
Melezitose	-	-	-	-	-	-	-
Arabinose	-	-	_	_	-	_	-
Cellobiose	-	-	_	_	_	_	_
Fructose	-	-	_	_	_	_	_
Galactose	-	_	_	_	_	_	_
Glucose	-	+	+	+_	_	+	_
Lactose	-	-	_	_	+	_	+
Maltose	-	_	+	+	_	+	_
Mannitol	-	+	+	+	+	+	+
Mannose	-	V	+	+	+	+	+
Melibiose	-	+	_	_	+	_	+
Raffinose	-	+	+	+	_	+	_
Rhamnose	-	+	_	_	+	_	+
Ribose	-	_	_	_	—	_	_
sucrose	-	+	_	_	_	_	_
Salicin	+	_	_	_	_	_	_
Sorbitol	-	_	_	_	_	_	_
Trehalose	+	_	+	+_	_	+_	_
Xylose	_	_	_	_	+		+_
		_	_	_	_		

_: Negative Reaction, +: Positive Reaction, V: Variable Reaction.

Acidification Ability

The results of acid production of the 07 isolated of LAB revealed that 03 isolates of *Lactobacillus* (LB_1.LB_2 .LB_3) was found to be fast acid producers belong to reduce the PH of the skim milk medium from an initial PH (6.71),(7.11) and (7.20) to a final value of (4.6).(4.38) and (4.73) respectively (figure1) in 4h and a final titratable acidity value (70D°),(75D°),(69D°) respectively with the same period (figure 2). Whereas, one strain of *Streptococcus* (ST1) considered as medium acid producer belong to the drop their PH from an initial value (6.77) to a final PH 4.6 within 48h (figure2).they also attained the titratable acidity (56D°) at 48h of incubation. The *Enterococcus* species (E_1),(E_2) showed a slow acid production ability as it reduced the initial PH (6.77),(6.56) to a final PH (4.53),(4.33) about 72h of fermentation and attained final titratable acidity values of (43D°),(49D°) (figure2). These results are similar to that reported by authors such as (Hassaine, Zadi-Karam, & Karam, 2007) whose indicated that the *Enterococcus* didn't reducer the PH of milk to (5,0) after 24 h incubation. The decreasing of the PH during the process of fermentation of our strains has benefices such as prevention and inhibition for pathogenic flora.

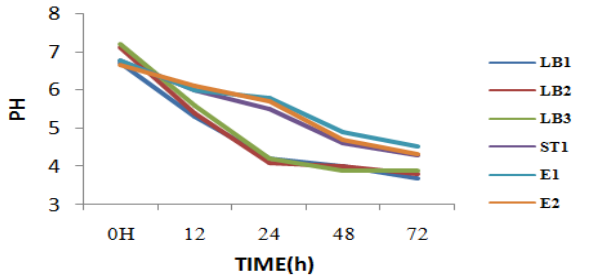

Figure 1: Change The Ph In Skim Milk of Lactic Acid Bacteria Acidity Incubated In 42C°. (LB1:*Lacobacillusdelbrueckiisubsp.bulgaricus,* LB2*: Lactobacillus delbrueckii Subsp. lactis.* LB3: *Lactobacillus Casei subsp.rhamnosus.* ST1: *Streptococcus thermophilus* E1: *Enterococcus faecalis, E2: Enterococcus faecium)*

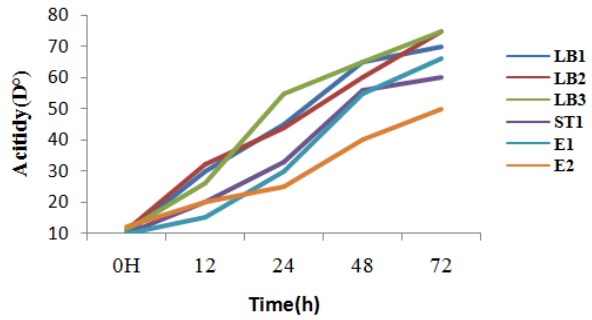

Figure 2: Evolution for Acidity En (D°) in Skim Milk of Lactic Acid Bacteria Acidity Incubated in 42C°. (LB1: *Lacobacillus delbrueckii subsp bulgaricus.* LB2*: Lactobacillus delbrueckii Subsp. lactis*. LB3: *Lactobacillus Casei subsp .rhamnosus.* ST1: *Streptococcus Thermophilus* E1: *Enterococcus faecalis.* E2: *Enterococcus faecium).*

Activity Proteolytic:

The proteolytic activity is very important factor in the development of organoleptic properties of different dairy products. Proteolysis could also contribute to preventing allergies frequent in Children less than 3 years of age due to poor digestion of milk proteins (Pescuma et al., 2009)**.** The production of good quality fermented dairy products is dependent on proteolytic properties of the starter bacteria, since peptidase and amino acids formed during fermentation have a direct

impact on flavor development, or serve as flavor precursors in dairy products (Seifu et al., 2012) In my test all strains characterized as positive proteolytic except the strain E2 with different diameter as reported in (Table 3). The *Lactobacillus* considered as higher quality compared with *Streptococcus* an *Enterococcus.*

Table 3: Activities proteolytic of LAB with Evaluation of zone of hydrolyze

Strains	Codes	Diameter (mm)	Evaluation	
Lacobacillus delbrueckii subsp bulgaricus,	LB1	20	+++	Goode
Lactobacillus delbrueckii Subsp. lactis	LB2	22	+++	Goode
Lactobacillus Casei subsp rhamnosus	LB3	34	+++	Goode
Streptococcus thermophilus	ST1	14	++	Meduim
Enterococcus. *faecalis*	E1	10	+	Wake
Enterococcus faeceium	E2	0	-	Negative

—: zone of proteolytic activity a diameter: ϕ = 0mn.
+: zone of Proteolytic activity whose diameter is between: ϕ =5-15mm.
+ +: zone of Proteolytic activity whose diameter is between: ϕ =10-20mn.
+++: zone of Proteolytic activity whose diameter ≥ 20mm (Cheriguene et al., 2007)

Antimicrobial Activity

The results of antimicrobial activity were shown in (figure 3). The tested isolated indicated different levels of inhibitory action against of pathogenic strains. 05 isolated were demonstrated an inhibitory activity against four pathogen strains. With different zone of inhibition. the most inhibition isolated was showing by the LB_1 against *E coli*, *Staphylococcus aureus* and *Listeria monocytongne* with an inhibition zone (5.5mm),(4.5mm), (4.9mm) respectively suited by ST_2 with (4 mm), (3mm), (3.8mm) respectively and wake inhibition for *Enterococcus* with (2mm),(2.2mm) for *Enterococcus faecalis*, negative for *Enterococcus faecium*. This results are similar than other authors like (Marion et al., 1997), Where indicate the power of inhibition action of *Lactobacillus.*

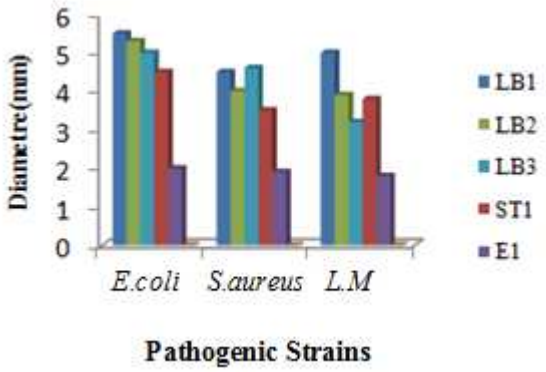

Figure 3: The Zone Inhibition of LAB Against Pathogenic Strains
(LB1: *Lactobacillus Delbrueckii Subsp. Bulgaricus.* LB2*: Lactobacillus Delbrueckii Subsp. Lactis.*
LB3: *Lactobacillus Casei Subsp. Rhamnosus.* ST1: *Streptococcus thermophilus*
E1: *Enterococcus faecalis* .E2:*Enterococcus faecium)*

CONCLUSIONS

Our study revealed that dairy milk of Algeria has high potential of lactic acid bacteria including in various fermentation. The dominants thermophilic acid lactic bacteria in various fermented milk were *Lactobacillus* (10%), *Enterococcus* (58%), and *Streptococcus* (8%). in order to use as starter cultures in manufactures fermented milk, we should to focus in future research for desirable characteristics such as acidity, the production of Exopolysaccharides and Bacteriocins and Aroma. Many studies confirmed that the ability of LAB to inhibit the other strains pathogen in dairy

products with beneficial effects on human health.

ACKNOWLEDGEMENTS

The authors thank the all persons helping in order to complete this investigation.

REFERENCES

1. Aslim, B., Yuksekdag, Z. N., Sarikaya, E., & Beyatli, Y. (2005). Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products. *LWT - Food Science and Technology*, *38*(6), 691–694. https://doi.org/10.1016/j.lwt.2004.08.001
2. Attia, H., Kherouatou, N., & Dhouib, A. (2001). Dromedary milk lactic acid fermentation: microbiological and rheological characteristics. *Journal of Industrial Microbiology and Biotechnology*, *26*(5), 263–270.
3. Beerens, H., & Francois, M. (1990). *Gu{'\i}a pr{á}ctica para el an{á}lisis microbiol{ó}gico de la leche y los productos l{á}cteos*. Zaragoza, ES: Acribia.
4. Bettache, G., & Mebrouk, K. (2004). Characterization of lactic acid bacteria isolated from Algerian arid zone raw goats milk. *African Journal of Biotechnology*, *3*(6), 339–342. https://doi.org/10.5897/AJB2004.000-2062
5. Burns, P., Patrignani, F., Serrazanetti, D., Vinderola, G. C., Reinheimer, J. A., Lanciotti, R., & Guerzoni, M. E. (2008). Probiotic Crescenza cheese containing Lactobacillus casei and Lactobacillus acidophilus manufactured with high-pressure homogenized milk. *Journal of Dairy Science*, *91*(2), 500–512.
6. Cheriguene, A., Chougrani, F., Bekada, A. M. A., El Soda, M., & Bensoltane, A. (2007). Enumeration and identification of lactic microflora in Algerian goats milk. *African Journal of Biotechnology*, *6*(15).
7. Hassaine, O., Zadi-Karam, H., & Karam, N.-E. (2007). Technologically important properties of lactic acid bacteria isolated from raw milk of three breeds of Algerian dromedary (Camelus dromedarius). *African Journal of Biotechnology*, *6*(14).
8. Hassaïne, O., Zadi-Karam, H., & Karam, N. E. (2008). Phenotypic identification and technological properties of lactic acid bacteria isolated from three breeds dromedary raw milks in south Algeria. *Emirates Journal of Food and Agriculture*, *20*(1), 46–59.
9. Khedid, K., Faid, M., Mokhtari, A., Soulaymani, A., & Zinedine, A. (2009). Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. *Microbiological Research*, *164*(1), 81–91.
10. Marion, D. W., Penrod, L. E., Kelsey, S. F., Obrist, W. D., Kochanek, P. M., Palmer, A. M., … DeKosky, S. T. (1997). Treatment of traumatic brain injury with moderate hypothermia. *New England Journal of Medicine*, *336*(8), 540–546.
11. Pescuma, M., He bert, E. M., Dalgalarrondo, M., Haertle , T., Mozzi, F., Chobert, J.-M., & de Valdez, G. (2009). Effect of Exopolysaccharides on the Hydrolysis of $β$-Lactoglobulin by Lactobacillus acidophilus CRL 636 in an in Vitro Gastric/Pancreatic System. *Journal of Agricultural and Food Chemistry*, *57*(12), 5571–5577.
12. Samelis, J., Maurogenakis, F., & Metaxopoulos, J. (1994). Characterisation of lactic acid bacteria isolated from

naturally fermented Greek dry salami. *International Journal of Food Microbiology*, *23*(2), 179–196.

13. Schillinger, U., & Lucke, F. K. (1987). Lactic-acid bacteria on vacuum-packaged meat and their influence on shelf-life. *Fleischwirtschaft*, *67*(10), 1244–1248.
14. Seifu, E., Abraham, A., Kurtu, M. Y., & Yilma, Z. (2012). Isolation and characterization of lactic acid bacteria from Ititu : Ethiopian traditional fermented camel milk. *Journal of Camelid Science*, *5*, 82–98.
15. Steel, R. G. D., Torrie, J. H., & DICKEY, M. (1980). A biometrical approach. *Principles and Procedures of Statistics*, 8–566.
16. Terzaghi, B. E., & Sandine, W. E. (1975). Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiology*, *29*(6), 807–13. https://doi.org/10.1.1.88.2825
17. Zhu, W. M., Liu, W., & Wu, D. Q. (2000). Isolation and characterization of a new bacteriocin from Lactobacillus gasseri KT7. *Journal of Applied Microbiology*, *88*(5), 877–886. https://doi.org/10.1046/j.1365-2672.2000.01027.x