

Cultivable Lactic Acid Bacteria Isolated from Algerian Raw Goat's Milk and Their Proteolytic Activity

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Abstract: The proteolytic systems play an essential role in nitrogen metabolism of lactic acid bacteria in milk. The extracellular cell wall-bound proteinase is a key enzyme in this system, in which its activity is necessary for the growth of lactic acid bacteria in milk by initiating the breakdown of casein to smaller peptides. The screening of the proteolytic strains has been achieved on three solid media YMA, PCA and FSDA. The strain of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis*, biovar. *diacetylactis* and the *Enterococcus* were tested. *Lactococcus lactis* produced a large hydrolysis zone more than 10 mm of diameter. The concentration of 3 and 5% of inoculum produced the highest acidity which was superior to 60°D. The proteolytic strains of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* at 3% of inoculum showed a maximal acidification rate 5°D/h and 0.3 unit pH/h. The strongest proteolytic strain *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* produced an acidification rate of 8°D/h and a pH rate which decreases to 0.28 unit of pH/h with a final pH value of 4.29. The rate of casein hydrolysis by the original *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (90 mg h⁻¹) was three times higher. The proteolytic activity of this strain can be exploited for the selection of performante lactic acid bacteria for the Algerian dairy industry.

Keys words: Lactic acid bacteria • proteolysis • caseins • goat's milk

INTRODUCTION

The primary role of lactic acid bacteria is the production of lactic acid from carbohydrates, resulting in a pH decrease and proteolysis as to liberate short peptides and free amino acids. These compounds affect flavour and texture of products [1]. The proteolytic system of lactic acid bacteria consists of a cell envelope associated proteinase, specific peptide and amino acid transport systems and several cytoplasmic peptidases. An important metabolic function that influence the fast growth of lactococci in milk is their proteolytic system, which is required to obtain the amino acids needed for growth to high cell density [2]. Mills and Thomas [3] reported that the concentration of amino acids especially isoleucine and leucine is very low in milk. However, the lactococcal proteinase remains the only proteolytic enzyme whose extracellular localization is ascertain. The type of proteinase was shown to play a crucial role in the interaction between proteolytic and nonproteolytic

lactococci associatively cultured in milk [4-6]. A proteinase, located at the outer surface of the cell catalyses partial hydrolysis of one or more of the casein components of bovine milk to a number of oligopeptide products [7].

The selection of new strains of lactic acid bacteria for technological interest and the exploitation of their potentialities is a subject to their growth capacity in milk. The objective of this study is to select some strains of *Lactococcus* sp which have a high proteolytic activity and susceptible to be integrated as a starter in fermented milk. The microbiological methods used here permitted to classify the *Lactococcus* sp strains by their proteolytic activity levels.

MATERIALS AND METHODS

Bacterial strains and growth conditions: Lactic acid bacteria strains were isolated from Algerian raw goat's milk and they belong to the culture collection of Applied

Microbiology Laboratory, Department of Biology, Sciences Faculty, Oran University Algeria, were used in this study. 32 strains of lactic acid bacteria isolates are listed in Table 1. All strains were stored at -80°C in a reconstituted skimmed milk containing 30% (wt/vol) glycerol. For cultivation, aliquots were reactivated in M17 broth at 30°C for 24 h [8]. Cultures were stored at 4°C and maintained in M17 medium every month.

Strains selection: After activation of the cultures in M17 broth, the purification was achieved in M17 agar medium and incubated at 30°C for 24 h. For the tests of proteolytic activity, strains were grown on three types of media containing 1% of sterile skimmed milk: The solid media Plates Count Agar (PCA), Yeast Milk Agar (YMA) and FSDA agar were used [9, 10]. The plates were evaluated by measuring the diameter of the hydrolysis and clear zones formed around the colonies which served into classifying the strains according to their proteolytic activity. In all cases, the average value from three replicates and standard deviation were calculated.

Proteolytic strains identification: Strains were identified as to possess the proteolytic activity and they were subsequently characterized according to the criteria described by Badis *et al.* [10]. Strains were subcultured in M17 broth, incubated at 30°C until visible growth occurred. Strains were checked for their purity by streaking colonies on M17 agar. Plates with pure culture were used to rapidly test for cell morphology by microscopy, Gram and catalase reaction. Gram-positive and catalase negative strains were further identified by a limited number of biochemical and physiological tests: gaz production, arginine hydrolysis on M16 BCP agar [11], which differentiates between the strains of the *Leuconostoc* genus and the heterofermented *Lactobacillus* genus. Citric acid degradation on solid medium of Kempler and Mac Kay [12] separated the subspecies of *Lactococcus*. The MSE agar medium [13] differentiates the subspecies of *Leuconostoc* and the Sherman test used in order to differentiate the *Streptococcus* and the *Lactococcus* species. Other tests were applied such as, the growth in salt, pH 9.6 broth and thermoresistance as to separate the *Enterococcus* sp. [10, 14].

The fermentative profil of several carbohydrates (glucose, lactose, xylose, fructose and sucrose) was carried out [15-17].

Optimization of proteolysis detection on solid media: Lactic acid bacteria which were cultured by multipoint

system on the surface of three different media PCA, YMA and FSDA containing various concentrations of skimmed milk (1, 2 and 4% w/v) which serve in evaluating the proteolytic activities of the strains. Hydrolysis proteolytic zones of the strain were compared and statistical analysis was carried out. The calculation of the difference between two observed average measurement were compared to the standard variance, using the T Student test [9].

Strains which showed positive proteinase phenotypes on assays were grown on 11% sterilized skimmed milk medium which was sterilized by autoclaving at 110°C for 10 min. Acidifying activity of strains was measured according to the International Dairy Federation (IDF) standard 306 [18], Kihal *et al.* [19] and Alonso-Calleja *et al.* [20]. The dornic acidity as well as the pH were measured every 2 h.

Sterilized milk was inoculated at 0.2% with each pre-cultured strain in MRS broth at 30°C for 24 h, in order to obtain approximately 10^6 cfu ml⁻¹ and then were incubated at 30°C for 24 h. The proteolytic activity of the strains was determinate by the tyrosine method, according to the International Dairy Federation (IDF) standard 149A [18].

After incubation until coagulation of milk at 30°C, the culture was inoculated in 200 ml of sterilized skimmed milk and homogenized. The mixture was distributed in tubes and the kinetics of growth and acidification were measured every 2 h. Determination of the specific growth rate μ and the calculation of some arithmetic parameters permitted to get values of the differences existing in the results [19].

Determination of caseolytic activity: Kinetic of growth in presence of casein: The proteolysis of casein was determinate in M17 broth medium containing 1 g l⁻¹ of casein as a substrate: 10 ml of 18 h freshed culture M17 broth was centrifugation (4000 rpm during 10 min at 4 °C). The Cell pellets were collected and washed twice in tryptone salt. They served to inoculate 100 ml of M17 casein broth. The growth of all strains was measured every hour by optical density at 600 nm by spectrophotometer. Casein residue was measured according to Bradford method [1].

RESULTS AND DISCUSSION

Strains characterization: All strains were gram positive and had a cocci form in pair or chains. A 28 of 32 purified strains are proteolytic giving clear visible zone on PCA and YMA +2% skimmed milk medium respectively. Morphological, physiological and biochemical

Table 1: Identification of lactic acid bacteria strains

Strain code	Identification
La12, La15, La16, La32, La34, La36, La38, Ma4, Ma6, Ma18, Ma19, Ma21, Ma26, Ma37, Max, SLO3+, LcX1	<i>Lactococcus lactis</i> subsp. <i>lactis</i>
La4, La20, La21, La23, La24, La30, La35, Ma20, Ma24, Ma25, SD17	<i>Enterococcus</i> sp.
La11, Ma1, Ma2, Ma27	<i>Lactococcus lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i>

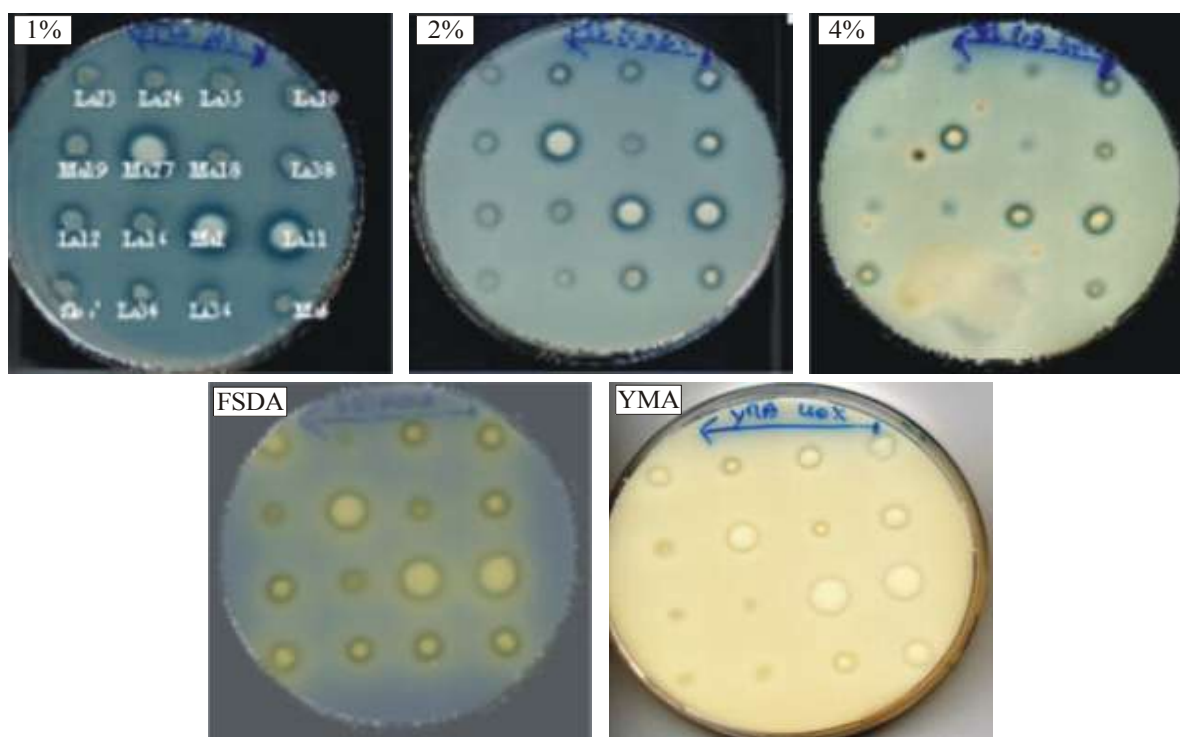


Fig. 1: Optimization of the detection of proteolysis on PCA medium (1, 2 and 4% of skimmed milk) on FSDA medium and YMA medium

characteristics [10, 12, 21] allowed the identification of: 17 strains which belongs to *Lactococcus lactis* species, 11 strains belonging to the genus *Enterococcus* and 4 species belonging to *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (Table 1).

Proteolytic activity: Four strains La11, Ma1, Ma2 and Ma27 have presented a proteolytic activity which raised on the three tested concentrations of milk and also on the two solid media culture PCA (Fig. 1) and YMA. On PCA medium, milk concentrations resulted in the appearance of a hydrolysis zone whilst a significant difference was observed between the presence of the concentrations (1 and 4%) of skimmed milk however, it was highly significant in the presence of 1 and 2% of skimmed milk. On YMA medium, the difference was highly significant between 1% and the other two concentrations of skimmed milk therefore 1% of skimmed milk is a suitable

concentration for detecting proteolytic activity in lactic bacteria strains. On FSDA medium, in addition to the proteolytic activity, the use of lactose and the production of acid lactic were also ascertained by the turn of bromocresol purple colour indicator to Yellow as shown in (Fig. 1).

Most of these strains yielded most visible zones with diameters varying between 0.15mm to 11.5mm which were established in the presence of 1% of skimmed milk. All strains have presented a hydrolysis zone of 11mm diameter and which was identical to La11, Ma1 and Ma27 strains (11 mm).

Statistical evaluation of strains activity: Screening for hydrolysis zones produced by several proteolytic strains were achieved on three different media and as a result a hydrolysis zones of 5mm to 10mm of diameter were produced by 73% of strains on FSDA medium, 70% on

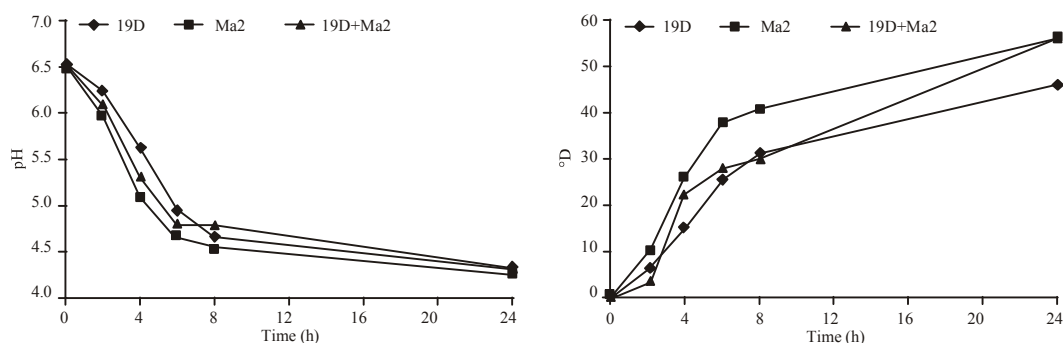


Fig. 2: Kinetics of pH and dornic acidity evolution in pure and mixed culture on skimmed milk

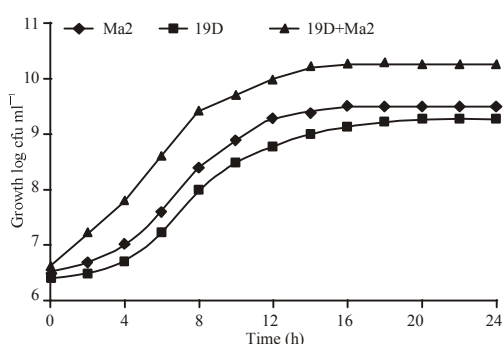


Fig. 3: Kinetics of growth of the 19D and Ma2 strains in pure culture and mixed culture

PCA medium and 59% on YMA medium. However, strains producing hydrolysis zones which their diameters higher than 10 mm was represented by 15.38% on FSDA, only 3.7% on PCA medium and 7.4% on YMA medium. Therefore, from these observations we can postulate that FSDA is the most favourable medium for detecting proteolytic lactic acid bacteria.

Moreover, proteolysis zone of diameter higher than 1 cm was represented in 7.5% of the strains on YMA medium and it reached 15.38% on FSDA medium and this led to differentiate between the strongly and slightly proteolytic strains [9, 22].

pH kinetic on skimmed milk: The inoculum concentration of 3 to 5% of strain (Ma2) which was incubated for 24 h in skimmed milk produced the highest acidity which was superior to 60°D however, the latter decreased to 35°D with an inoculum concentrations that varied between 0.1 and 0.5%. Moreover, the same phenomenon was observed with concentrations of 3 and 5% of inoculum incubated in skimmed milk for only 6 h whilst the pH decreased rapidly (inferior than 5). Therefore, the production of lactic acid is related to the concentration of the initial inoculum.

A maximal speed of 5°D per hour is obtained while using a concentration of 2% of inoculum, this speed rate is steady even if the concentration of the inoculum gets higher however the former gets lower than 5°D/h if the concentration decreases to less than 2%. The fall of velocity of the pH was about 0.35 Unit per hour for Ma2 strain.

In order to follow the kinetics of growth on milk medium and M17 synthetic medium Dudley and Steele [23] used an inoculum of 1% and in order to study the kinetic of caseins degradation [24], which is going to be in relation with the strain used. Demarigny *et al.* [25] obtained a maximum pH rate which varied between 0.34 and 0.51 Unit of pH/h using the same concentration of inoculum 1%. Kinetic of acidity for 12 strains varied between 0.75 and 3.5°D per hour and pH decreases from 0.03 to 0.14 Unit of pH per hour, final dornic acidity and pH were of 35°D and 4.63 in strains LeX1 respectively.

Strain Ma2 produced a velocity of acidity of 8°D/h and a varied velocity of pH of 0.28 Unit of pH/h, the maximum rate of the produced acidity is 51°D with a final pH of 4.29. Juillard *et al.* [4] obtained a final pH of 4.5 after 9 h of growth for *Lactococcus lactis* which is identical to Ma2 (*diacetylactis*). strain used in this work. *Lactococcus lactis* subsp. *cremoris* studied by Ruas-Madiedo *et al.* [26] achieved a final pH of 4.4 on milk medium. Ma2 strain had showed both good kinetic acidity and growth on milk medium.

La38 is a weak proteolytic strain which is characterised by a weak pH variation in relation with a weak dornic acidity evolution. Maximal velocities for pH and acidity are of 0.0475 Unit of pH per hour and 3.75°D/h and a final value of pH 24°D was achieved after 24 h of incubation.

Growth kinetic in mixed culture: *Lactococcus lactis* (Ma2) and *Leuconostoc mesenteroides* (19D) strains have produced on a pure culture (5% of inoculum) a final acidity of 56 and 46°D, respectively. On the mixed culture,

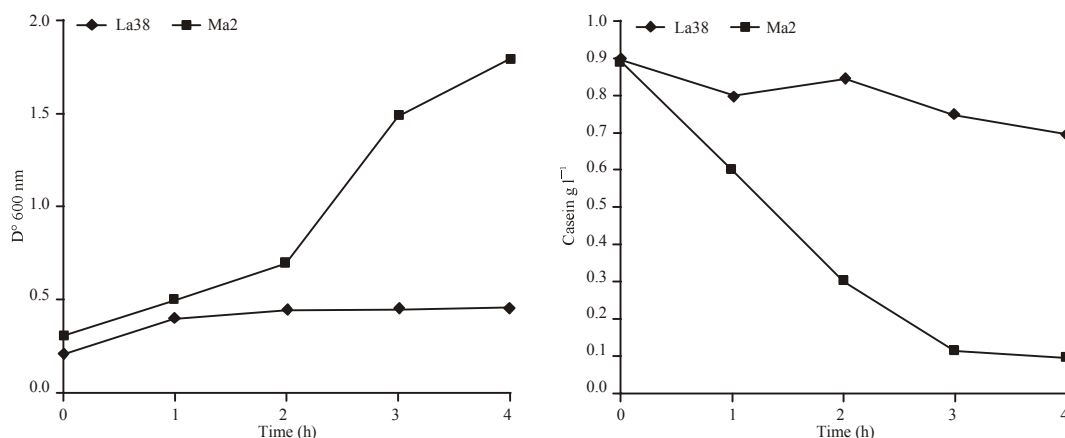


Fig. 4: Growth of La38 and Ma2 strains on M17 casein broth medium incubated at 30°C

the dornic acidity remained stable and identical to the one produced by *Lactococcus lactis* (56°D) (Fig. 2). pH variations followed the same speed of dornic acidity in pure and mixed culture on milk medium. Ma2 and 19D strains produced maximum velocity of 0.32, 0.33 and 0.32 in pure and mixed cultures respectively. Moreover, maximum velocities obtained for the variation of dornic acidity is of 7°D/h for Ma2 strain and 5°D for 19D strains in mixed culture.

Curve obtained for the weak proteolytic strains were characterized by a weak velocity of milk acidity; however, the inverse was obtained with the proteolytic strains. In the mixed culture, samples that have been studied did not show any relation neither with synergy or antagonism.

In milk medium, the kinetics showed that the pure cultures of *Leuconostoc mesenteroides* (19D) and *Lactococcus lactis* (Ma2) have a growth rate of 0.49 h⁻¹ and 0.12 h⁻¹ respectively; however, it is slightly higher (0.53h⁻¹) in the mixed culture (Fig. 3). In MRS or M17 medium, this rate is practically identical in *Leuconostoc mesenteroides* species due to the richness of the medium with nutritive elements [27].

The same rate (0.24 h⁻¹ to 0.4 h⁻¹) was obtained with *Bifidobacterium species* which are originally from human, cultivated in skimmed milk medium [28] and also with *Lactococcus sp.* (0.32 to 0.48 h⁻¹) [29].

We have noted a different behaviour within the chosen species, while exponential phase for both, pure culture of *Leuconostoc mesenteroides* and the mixed culture (19D and Ma2), is accelerated between 2 and 6 h of incubation and this is due to the effect of citrate on the stimulation of *Leuconostoc mesenteroides* species [19]. *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* culture had presented a regular growth whilst the exponential phase is not accelerated.

Kinetic growth on M17 casein medium: Hence La38 strain hardly bear casein (rate of 0.564 h⁻¹) the Ma2 strain had developed rapidly (rate of 1.162 h⁻¹). *Lactococcus* strain's behaviour towards casein [30, 31] can be referred to the cell wall bound protease which is responsible for casein degradation into amino acids and peptides [5]. In addition, the rapid development for Ma2 strain in the presence of caseins is due to its enzymatic equipment.

Dosage of residual casein: The Velocity average of the consumption of casein (90 mg h⁻¹) by *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (Ma2) is three times higher than the one consumed by *Lactococcus lactis* subsp. *lactis* (La38) (23.3 mg h⁻¹). Final concentrations of casein residues are 0.1 and 0.54 g l⁻¹ in Ma2 and La38 strains respectively, which is identical to other studies [9].

CONCLUSIONS

The effect of culture media on hydrolysis zones of casein by *Lactococcus* chosen species is weak; however skimmed milk concentrations had a remarkable influence.

The majority of strains (more than 60%) produced hydrolysis zones of 5mm to 10mm of diameter. The proportion of *Lactococcus lactis* species which posses hydrolysis zones superior than 10mm was inferior than 10%. Among strongly proteolytic strain, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* strain gave a velocity of acidity of 8°D/h and a varied pH velocity of 0.28Unit of pH/h and the final pH was 4.29.

Leuconostoc mesenteroides and *Lactococcus lactis* had growth rate of 0.49 and 0.12 h⁻¹ in pure culture of milk media, however, their growth was a bit higher (0.53 h⁻¹) in mixed culture.

The growth of *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* and the slightly proteolytic strain *Lactococcus lactis* subsp. *lactis* on M17 casein medium, have showed that the first strain developed rapidly with a rate of 1.162 h^{-1} ; however, it was weak in the case of the second strain (0.564 h^{-1}) [25].

The results obtained suggest that lactic acid bacteria found in goat's raw milk can be used in dairy industry by their proteolytic and acidifying activities. Our results have also shown that a rich source of strain variability exists in the proteolytic activities. Goats' milk may, therefore, constitute a source of strains with physiological properties of biotechnological interest.

Further studies should be directed on the identification and characterization of the peptides that are accumulated in the milk after the various strains application which play an important role in the proteolytic enzymes activities. The choose of used strain could be an instrumental knowledge in the improvement of the organoleptic quality of fermented dairy products.

REFERENCES

- Meijer, W.C. and J. Hugenholtz, 1997. Proteolytic enzyme activity in lactococci grown in different pretreated milk media. *J. Applied Microbiol.*, 2: 139-146.
- Pelaez, C. and T. Requena, 2005. Exploiting the potential of bacteria in the cheese ecosystem. *Int. Dairy J.*, 15: 831-844.
- Juillard, V., E.R. Bars, E.R.S. Kunji, W.N. Konings, J.C. Gripon and J. Richad, 1995. Oligopeptides are the hand source of nitrogen for *Lactococcus lactis* during growth in milk. *Applied Environ. Microbiol.*, 8: 3024-3030.
- Flambard, B., S. Helinck, J. Richard and V. Juillard, 1998. The contribution of caseins to the Amino Acid supply for *Lactococcus lactis* depends one the standard of cell envelope protéinase, *Applied Microbiol.*, 4: 1991-1996.
- Garault, P., C. Letort, D. Juillard and V. Monnet, 2001. The biosynthesis of the amino acids with connected chain and purins: Two essential ways for an optimal growth of *Streptococcus thermophilus* in milk. *Milk*, 81: 83-90.
- El Soda, M., 1993. The role of lactic acid bacteria in accelerated cheese ripening. *FEMS. Microbiol. Rev.*, 12: 239-252.
- Mathot, A.G., M. Kihal, H. Prevost and C. Diviès, 1994. Selective Enumeration of *Leuconostoc* on Vancomycine Agar Media. *Intl. Dairy J.*, 5: 459-469.
- Huggins, A.R. and W.E. Sandine, 1984. Differentiation of fast and slow fox trot milk-coagulating isolates in strains of *lactic streptococci*. *Dairy Sci.*, 67: 1674-679.
- Badis, A., D. Guetarni, B. Moussa-Boudjemaa, D.E. Henni and M. Kihal, 2004. Identification and technological properties of lactic acid bacteria isolated from raw goat's milk of Algerian races. *Food. Microbiol.*, 21: 579-588.
- Thomas, T.D., 1973. Agar medium for differentiation of *Streptococcus cremoris* from the other bacteria. *NZ J. Dairy. Sci. Technol.*, 8: 70-71.
- Kempler, G.M. and L.L. McKay, 1980. Improved medium for detection of citrate-fermenting *Streptococcus lactis* subsp. *diacetylactis*. *J Applied Environ. Microbiol.*, 39: 956-927.
- Mayeux, J.V., W.W.E. Sandine and P.R. Elliker, 1962. With selective medium for detecting *Leuconostoc* organisms in mixed strain choke cultures. *J. Dairy Sci.*, 45: 655-656.
- Mathara, J.M., U. Shillinger, P.M. Kutima, S.K. Mbugua and W.H. Holzapfel, 2004. Isolation, identification and characterization of the dominating microorganisms of Kule naoto: The Maasai traditional fermented milk in Kenya. *J. Food. Microbiol.*, 3: 269-278.
- Durlu-Ozkaya, F., V. Xanthopoulos, N. Tunail and E. Litopoulou-Tzanitaki, 2001. Technologically important properties of lactic acid bacteria isolates from raw ewes' milk. *J. Applied Microbiol.*, 91: 861-870.
- Mannu, L., G. Riu, M. Comunian, C. Fozzi and F. Scientu, 2002. A preliminary study of lactic acid bacteria in whey choke culture and industrial *piconiro sardo* ewes milk cheese: Pcr-identification and evolution during repining. *Int. Dairy J.*, 12: 17-26.
- Guessas, B. and M. Kihal, 2004. Characterization of lactic acid bacteria isolated from Algerian arid zone: Raw goat's milk. *Afr. J. Biotechnol.*, 3: 339-342.
- International Dairy Federation (IDF), 1997. Levains lactiques de cultures de bacteries lactiques. Norme de composition. IDF Standard 149A. Brussels, Belgium.
- Kihal, M., H. Prevost, M.E. Lhotte, D.Q. Huang and C. Divies, 1996. Instability of plasmid-encoded citrate permease in *Leuconostoc*. *J. Applied Microbiol.*, 22: 219-223.
- Alonso-Calleja, C., J. Carballo, R. Capita, A. Bernardo and M.L. Garcia-Lopez, 2002. Comparison of acidifying activity of *Lactococcus lactis* subsp. *lactis* strains isolated from goat's milk and valdeteja cheese. *Lett. Applied Microbiol.*, 34: 134-138.

21. Stiles, E. and H. Holzapfel, 1997. Lactic acid bacteria of foods and to their current taxonomy. Intl. J. Food. Microbiol., 36: 1-29.
22. Wehrle, K., N. Crowe, I. Van Boeijen and E.K. Arendt, 1999. Screening methods for the proteolytic breakdown of gluten by lactic acid bacteria and enzyme preparations. Eur. Food. Res. Technol., 209: 428-433.
23. Duddley, E.G. and J.L. Steele, 2001. *Lactococcus lactis* LMO 230 contains has single aminotransferase involved in aspartate biosynthesis, which is essential for growth in milk. Microbiol., 147: 215-224.
24. Herrero, M.A., J.M. Fresno, P.M.J. Gongalez and M.E. Tornadijo, 2003. Technological characters of lactic acid bacteria isolated from Armada cheese (Spanish goat's milk cheese hard). Int. Dairy J., 5: 469-479.
25. Demarigny, Y., V. Juillard, N. Deschanps and J. Richard, 1994. Comparison, on the practical level, of 3 models of study of the kinetics of lowering of the pH of the milk cultivated by *Lactococcus lactis*. Proposal of the concept "Vmar". Milk, 74: 23-32.
26. Ruas-Madiedo, P., A.C. Aling and P. Zoom, 2005. Effect of exopolysaccharides and proteolytic activity of *Lactococcus lactis* subsp. *Cremoris* strains one the viscosity and structure of fermented milks. Int. Dairy J., 15: 155-164.
27. Courtin, P., V. Monnet and F. Rul, 2002. Cell-wall proteinases prtS and prtB cuts A different role in *Streptococcus thermophilus/Lactobacillus bulgaricus* mixed cultures in milk. Microbiol., 148: 3413-3421.
28. Hadadji, M., R. Benaama, N. Saidi, D. Henni and M. Kihal, 2005. Identification of cultivable *Bifidobacterium* species isolated from breast-EDF infants faeces and to their technological properties for potential uses ace has probiotic cultures. Afr. J. Biotechnol., 4: 422-430.
29. Chambellon, E. and M. Yvon, 2003. Cod y Regulated Aminotransferases Ara T and β cat T play has major role in the growth of *Lactococcus lactis* in milk by regulating the internacellular pool of Amino Acids. Applied Microbiol., 3: 3061-3068.
30. Juillard, V., A. Guillot, D. Bars and J.C. Gripon, 1998. Specificity of milk peptide utilization by *Lactococcus lactis*. Applied Environ. Microbiol., 4: 1230-1236.
31. Parra, L., P. Fernandez de Plencia, V. Casal, T. Requena and C. Pelaez, 1999. Hydrolysis of β -casein (193-209) fragment by whole cells and fractions of *Lactobacillus casei* and *Lactococcus lactis*. J. Food Sci., 64: 899-902.2