Phenotypic identification of five homolactic-type lactic strains, isolated from three different barley-based biotopes

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Abstract- Lactic acid bacteria (LAB) are included in the groups of microbiota Beneficial; they are widely used in the field of biotechnology thanks to their technological and functional quality as well as nutritional. Accordingly, the objective of this study is the isolation of homolactic-type lactic acid bacteria from three different biotopes: barley with traditional lben, raw milk barley and fermented barley, having shown a technological property important including the ability to grow and adapt to different environments, this characterization is expressed by measuring the optical density at 600 nm using a spectrophotometer.
The isolated strains are purified on the solid MRS medium which is suitable for the specific search for lactobacilli. Initially, a pre-identification is carried out on the basis of the classic technique of microbiology which includes the study of morphological, physiological and biochemical character.
The phenotypic identification of the selected strains is then completed by the use of an API 50CHL Medium strip (Bio-Mérieux reference 50410) in the interest of making the results more reliable. Thus, the species is given automatically by the computer-aided database of "APIWEB" version (5.1).
The results obtained revealed that the five selected strains are identified as lactic acid bacteria that belong to the species: *Lactobacillus plantarum, Pediococcus spp,* and *Lactococcus lactis ssp lactis*.

Index Terms- Lactic acid bacteria, phenotypic identification, *Lactobacillus plantarum, Lactococcus lactis ssp lactis, Pediococcus spp.*

I. INTRODUCTION

Lactic acid bacteria synthesize their ATP through fermentation lactic **[1]** they are very diversified by their functional properties in biotechnology **[2, 3]** and characterized by their particular sugar metabolic activities. They are classified into two biochemical groups: the homolactic pathway, the final product of which is essentially lactic acid, and the heterolactic pathway, which produces in addition to lactic acid, other compounds such as ethanol, acetate, and CO2 **[4]**.

In this present work, we were interested in the isolation of lactic strains from: barley with traditional lben, barley with raw milk and fermented barley. The choice of strains is based on three steps. First, the pre-selection of isolated strains using a morphological study and a study on the presence of respiratory enzymes. Then, the selection is completed by physiological tests based on the effect of different environments on the growth of the selected strains, tolerance to low temperatures is a sought criterion for application in improved products **[5].** Finally, pre-identification at the genus stage is supported by biochemical tests. The identification of strains retained at the species stage is accomplished by studying the metabolism of hydrates of carbon in an API gallery consisting of 50 wells containing carbohydrates and their derivatives (heterosides, polyalcohols, uronic acids).

II. MATERIALS AND METHODS

II.1. Isolation and purification of lactic strains

A series of decimal dilutions is made from each sample **[6]** the seeding is done in depth, the isolation is carried out on the MRS medium (Man Rogosa and Sharpe) **[7, 8]**. Seeded Petri dishes are homogenized and incubated for 24 hours at 30°C **[9]**. Purification is performed by streaking **[10]** after a successive series of transplanting **[11]** until macroscopically and microscopically homogeneous and identical colonies are obtained. The strains are preserved on MRS agar medium which is tilted in test tubes at +4°C **[12].** A duplicate is stored in Eppendrof tubes containing MRS broth medium supplemented with 30%sterile glycerol at -20°C **[13, 14]**.

II.2. Phenotypic identification of selected lactic strains

A. Morphological study

Purified strains are examined by the first based test on the visual observation of the colonies directly with the naked

eye **[15]** followed by microscopic observation based on Gram stain using crystal violet, lugol, acetone alcohol, and fushin **[16, 17]** As for the second, the test consists of checking whether the strain has the catalase enzyme **[18]** cytochrome oxidase **[19]** and nitrate reductase **[15, 19]**.

B. Physiological study

The test is predicated on the search for a respiratory mode on VF agar (meat-liver) **[20]** completed by researching the ability to grow at different temperatures **[21, 22]** and at different NaCl concentrations **[21, 23]**. The growth of the lactic strainsis determined by measuring the optical density **[24]** using a type spectrophotometer (UV-2004 Power / 110/220V-50/60Hz). The reading is made at 600 nm against an uninoculated control.

C. Biochemical study

- Pre-identification at the genus stage

The test is based on fermentative type research which is interpreted by the presence or absence of gas production during glucose fermentation **[25, 26, 27]** then, the pre-identification is completed by the study of mobility on the Mannitol-Mobility medium **[28]**, investigation of the use of citrateas the sole carbon source on Simmons citrate agar **[29]** and the production of acid from the fermentation of various sugars (put according to their availability) in a classic tube gallery **[30]**.

- Identification at the species stage

The classification of the selected strains is made according to the ability to degrade the various carbon sources using the API 50CHL Medium gallery from Bio-Mérieux, France (reference 50410). The bacterial suspension is prepared from the harvest of young colonies (18 to 24 hours) by swabbing until a density at turbidity equal to that of the standard 2 McFarland standard is obtained. The latter is seeded on the CHL 50 medium prepared without glucose, without meat extract and added bromocresol purple as an indicator.
0.1 ml of inoculum is placed in the micro-tubes which contain 49 sugars **[31]**. The cupules of the gallery are covered with a thin layer of sterile paraffin oil to ensure anaerobic conditions. Then, they are deposited sterilely in the incubation box sprayed with sterile distilled water in the cells at the bottom to create a humid atmosphere. Incubation is done at 35°C ± 2°C. Thus, the reading is carried out at 24 and 48 hours. The identification of the species is given automatically thanks to a digital profile by the computer-aided database of "APIWEB" version (5.1) **[32].**

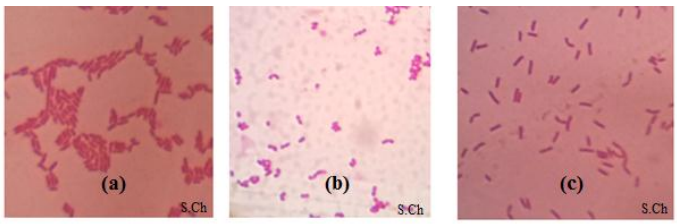
III. RESULTS AND DISCUSSION

Forty-two strains of lactic acid bacteria were obtained from three samples: barley with traditional lben, barley with raw milk, and fermented barley.
Beforehand, the study focused on the selection of lactic strains which present a metabolic activity of the homo-fermentative type. For this reason, twenty-two hetero-fermentative strains have been eliminated since they are capable of producing compounds other than lactic acid **[33]**, because they can damage the fermentation products through gaseous or alcoholic alterations. This interpretation is in agreement withthe work of Gänzle M.G **[34].** It is also noted that all the strains which presented a positivecatalase are discarded.
The second criterion sought is the ability to adapt to a certain environmental influence.Therefore, five lactic strains coded by (SC1, SC2, SC3, SC4, SC5) were the subject of this study. In addition, the phenotypic identification revealed that there are three species namely: *Lactobacillus plantarum*, *Pediococcus spp, Lactococcus lactis ssp lactis.* This taxonomy is synchronized with that of Klein G **[35]** and Stiles M.E **[36].**

- **Morphological, biochemical and physiological characterization of strains retained lactics**

First of all, the results of the morphological test showed that the appearance of the colony of the selected strains is round or lenticular in shape with different colors and varying sizes (photo 1). In addition, these strains are gram positive, immobile, of different shapes: bacillus, coccobacillus and cocci (photo2), and which possess neither nitrate reductase, nor cytoc hrome oxidase, nor catalase. These results are consistent with those of Mathialagan M **[37],** Asfour H.A **[38],** Gaspar P **[39],** Pringsulaka O **[40],** Ao X **[41],** and De Vos P **[42].**

Picture 1: Macroscopic appearance of three species of lactic acid bacteria isolated on MRS medium.



Picture 2: Microscopic observation of different form of lactic strains (MagnificationX100). (a) Cocobacillus

(b) Cocci (c) Bacillus.

The works of Coeuret V **[43]** assured that the determination of cell morphology is not sufficient to indicate the genus of the strain studied. Indeed, the biochemical analyzes presented in table 1 have illustrated that all the strains do not possess citrate-permease andproduce neither hydrogen sulphide nor CO2 during the fermentation of glucose. This makesit possible to appreciate the energy metabolism used by the strains studied, and to classify them in the groups of homo-fermentatives. These results are in line with those of Bennani S **[44],** Zhang S **[45]** and Thompson JK **[46].** It should be noted that they are also all capable of fermenting various sugars such as glucose, lactose, fructose, dextrose, galactose and mannose, while the fermentation of other sugars is variable from species to species.

Table 1: Results of the morphology and biochemical study of five selected strain

Characters studied		Lactic Strain				
		SC1	**SC2**	**SC3**	**SC4**	**SC5**
Colony shape		Round	Lenticular	Lenticular	Round	Round
Colony size		Small	Domed	Small	so small	Small
Color of the colony		Whitish with yellow reflection	Whitish with brown center	Whitish	Greyish	Whitish
Colony outline		Regular	Regular	Regular	Regular	Regular
Cell shape		Coco-bacillus	Bacillus	Cocci	Cocci	Cocci
Gram type		+	+	+	+	+
Catalase		-	-	-	-	-
Oxidase		-	-	-	-	-
Citrate utilization		-	-	-	-	-
Production of H_2S		-	-	-	-	-
Production of CO_2		-	-	-	-	-
Study of mobility		-	-	-	-	-
Fermentation type		H. F	H. F	H. F	H. F	H. F
Study of the fermentation profile of 15 sugars	Glucose	+	+	+	+	+
	Mannitol	+	+/-	+	+/-	+/-
	Sucrose	+	+/-	+/-	+/-	+/-
	Sorbose	+/-	+/-	+/-	+/-	+/-
	Amidon	+	+/-	+/-	+/-	+
	Lactose	+	+	+	+	+
	Arabinose	+/-	+/-	+	+/-	+
	Fructose	+	+	+	+	+
	Dextrose	+	+	+	+	+
	Maltose	+	+/-	+	+	+
	Xylose	+/-	+/-	+/-	+/-	+/-
	Raffinose	+	+	-	+/-	+/-
	Agarose	+/-	+/-	+/-	+/-	+/-
	Galactose	+	+	+	+	+
	Mannose	+	+	+	+	+

* (+) positive result, (-) negative result, (+/-) intermediate result, (H.F) homo-fermentative.

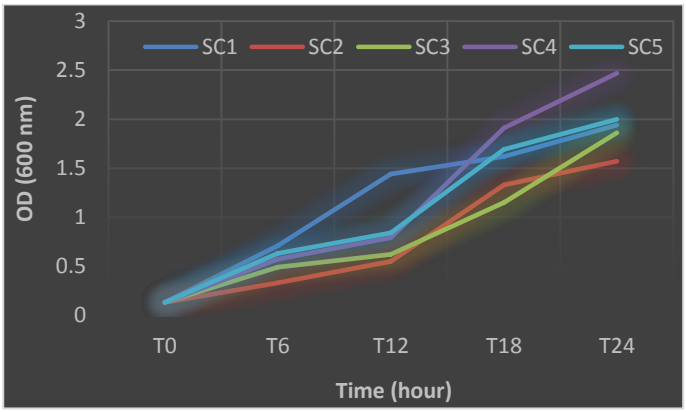
Furthermore, the results of the physiological study proved that all the strains are anaerobic but aerotolerant **[38]**. In addition, the property of tolerating certain environment is interpreted by the growth which appears in the tube as a homogeneous cloudiness and concentrated at the bottom in the liquid MRS medium **[47]**. Indeed, the values of the cell concentration set out in table 2 ensure that the five strains studied are resistant to salt stress since they are able to live in the presence of different concentrations of NaCl: 2%, 4% and 6%. Similarly, the cell density values presented in the same table show that these strains are also able togrow at different temperatures, namely: 15, 25, 35.45 and 55°C. This interpretation conforms to that of Badis A [**48]** and Samelis J [**14].**

However, the optimal growth temperature of all the strains studied is between 25°C and 35°C. On the other hand, low growth is marked at 15°C and 55°C. Though, the growth results announced at 45°C vary depending on the strain. Thus, better growth is recorded at 35°C. for all strains. Table 2 also illustrates this point. Therefore, according to the work of Yelnetty A [49] and Pelmont J [50] strains SC1, SC2, SC3, SC4, SC5 are classified in the group of mesophils.

Table 2: Influence of temperature and NaCl concentration on the growth of the strains studied.

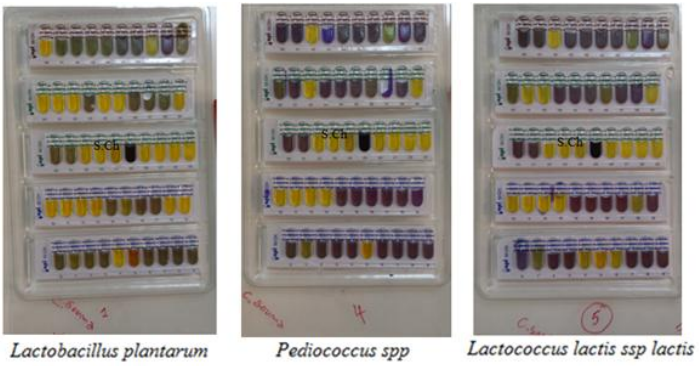
Parameters studied		Optical densityat 600nm after 48 hours of incubation				
		SC1	**SC2**	**SC3**	**SC4**	**SC5**
Growth temperature	15°C	0.33	0.37	0.20	0.18	0.36
	25°C	1.82	0.88	1.56	1.37	1.08
	35°C	1.94	1.57	1.86	2.47	2.00
	45°C	0.45	0.56	1.36	1.74	1.39
	55°C	0.22	0.30	0.21	0.19	0.18
NaCl concentration	2%	0.41	1.50	0.73	0.77	0.73
	4%	1.04	1.13	0.55	0.54	0.56
	6%	0.40	0.37	0.47	0.48	0.48

In this regard, the evolution of the growth of the five strains studied at 35°C is followed by the measurement of the optical density at 600 nm as a function of the incubation time per hour. The results of the latter are presented in curve 1.



Curve1: Monitoring of the evolution of strain growth (SC1, SC2, SC3, SC4, and SC5) at 35±2°C for 24 hours of incubation.

The five strains selected are classified on the basis of phenotypic properties, the results of the morphology, physiological and biochemical study make it possible to indicate that there are three genera, namely: *Lactobacillus*, *Pediococcus*, and *Lactococcus*, this taxonomy is identical withthat of Axelsson L **[51]** and Holzapfel W.H **[52].**
The results shown in photo 3 revealed that strains SC1, SC2, SC3, SC4, SC5 bind to the following species: *Lactobacillus plantarum* (99.8%), *Lactobacillus plantarum* (99.4%), *Lactococcus lactis ssp lactis* (82.7%), *Pediococcus spp* (87.8%), *Lactococcus lactis ssp lactis* (98.2%), successively. According to König H **[53],** De Almeida J **[54]** and Ceapa C **[55],** the study of the fermentation profile of sugars by the API 50 CHL gallery is considered as the basis for the identification of lactic acid bacteria. Furthermore, Ozgan D **[56]** also mentioned that this test guarantees the results.



Picture 3: Phenotypic identification of three species of lactic acid bacteria by the API 50CHLgallery.
(a) *Lactobacillus plantarum*, (b) *Pediococcus spp*,
(c) *Lactococcus lactis ssp lactis.*

IV. CONCLUSION

By way of conclusion, among the twenty homolactic strains selected, only five (5) strains isolated from: barley with traditional lben (SC1, SC2), raw milk barley (SC3, SC4) and fermented barley (SC5), which have ensured the desired physiological character, the ability to grow at a temperature of 25 ° C with better performance is a target parameter in the field of food biotechnology. Thus, this explanation is consistent with the work of **[5].**
Indeed, the identification by the study of phenotypic character gave: *Lactobacillus plantarum* (SC1, SC2), *Lactococcus lactis ssp lactis* (SC3), *Pediococcus spp* (SC4), and *Lactococcus lactis ssp lactis* (SC5). These species found are among the most important lactic acid bacteria applied in the food and pharmaceutical industries. In this direction, König H **[53]** and Bernardoa MP **[57]** assert this remark.

REFERENCES

[1] Pilet M, Magras C and Federigh M. (2005). Lactic acid bacteria. In: Food bacteriology (Federighi M). Ed: Economica, Paris, pp. 219-240.

[2] Saidi Y, (2020). Biodiversité de la microflore lactique du lait cru de dromadaire et évaluation de ses caractères technologiques. Université Oran1 Ahmed Ben Bella, Algerie. p 30.

[3] Florou-Paneri P., Christaki E., and Bonos E (2013). Lactic acid bacteria as source of functional ingredients lactic acid bacteria R&D for food, health and livestock purposes p 590-59.

[4] Mozzi, F., Raya, R., Vignolo, G. and Love, J. C (2016). Biotechnology of Lactic Acid Bacteria–Novel Applications, second edition.

[5] Fenster, K., Freeburg, B., Hollard, C., Wong, C., Laursen, R.R. and Ouwehand, A.C. (2019). The Production and Delivery of Probiotics: A Reviewof a Practical Approach. Microorganisms.7:83.

[6] Jerome, J. P., James, T. S., Stephen, L. (2004). Microbiologie. Ed. Dunod. Paris.P 479.

[7] Milani E, Shahidi F, Mortazavi S.A and Saeedi M, (2017). Isolation and identification of lactic acid bacteria in Kurdish cheese during ripening using 16S rRNA gene sequence analysis. Journal of food processing and preservation, 41(4), e13009.

[8] De Man, JC, Rogosa M, Sharpe, and ME (1960). A medium for the cultivation of lactobacilli. Journal of applied Bacteriology. 23: 130-135**.**

[9] Kacem, M. and Karam, N. (2006).Physicochemical and microbiological study of «Shmen», a traditional butter made from camel milk in the Sahara (Algeria): Isolation and identification of lactic acid.

[10] Zadi-Karam H., Kalbaza K., and Karam N.-E. (2011). Identification et caractéristiques technologiques de 18 souches de Leuconostoc isolées de lait de chamelle de Béchar. Rencontres autour des recherches sur les ruminants.

[11] Cheriguene, A., Chougrani, F., Bekada, A.M.A., El Soda, M., Bensoltane, A. (2007). Enumeration and identification of lactic microflora in Algerian goat"s milk. In African Journal of Biotechnology Vol. 6 (15), PP. 1854-1861.

[12] Badis A., Laouabdia-Sellami N., Guetarni D., Kihal M., and Ouzrout R. (2005). Caractérisation phénotypique des bactéries lactiques isolées à partir de lait cru de chèvre de deux populations caprines locales (Arabia et kabyle). 23: 30-37.

[13] Herrero M., Mayo B., Gonzalez B and Suarez J.E., (1996). Evoluation of technologically importantes traits in lactic bacteria isolated from spontaneous fermentation, J. Appl. Bacteriol., 81, pp. 565-570.

[14] Samelis J, Maurogenakis F, and Metaxopoulos J, (1994). Characterization of Lactic Acid Bacteria isolated from naturally fermented Greek dry salami. Inter.J. Food. Microbiol., 23: 179-196.

[15] Singleton, D.R., Powell, S.N., Sangaiah, R., Gold, A., Ball, L.M., and Aitken, M.D. (2005). Stableisotope probing of bacteria capable of degrading salicylate, naphthalene, or phenanthrene in a bioreactor treating contaminated soil. Appl. Environ. Microbiol. 71: 1202.

[16] Heleni S., Lefki P., Nikolao T. and Evanthia L.T., (2006). Populations, types and biochemical activities of aerobic bacteria and lactic acid bacteria from the air of cheese factories. Int. J. Dairy Technol. vol. 59, no3, pp. 200-208.

[17] Singleton P., (1999). Bacteriology. 4e Ed. Dunod, Paris p: 317.

[18] Bell C, Neaves P, and Williams AP, (2005). Food microbiology : laboratory practice. Blackwell publishing, USA.

[19] Mechai A., (2009). Isolement, caractérisation et purification de bactériocines produites par des bactéries lactiques autochtones : études physiologiques et biochimiques. University Badji mokhtar Annaba. Algeria, 160.

[20] Nehal F., (2007). Isolement et caractérisation de souches de *lactoccus lactis* à partir de différentes laits dans le périmètre du moyen cheliff. University Hassiba ben bouali chlef, p 44.

[21] Mokhbi, M. Kaid-Harche, K. Lamri, M. Rezki, and M. Kacem (2009), Selection of *Lactobacillus plantarum* strains for their use as starter cultures in Algerian olive fermentations. *Grasas yaceites*, 60 ; 82-88.

[22] Zadi karam H., and Karam N.E (2005). lactic acid bacteria from camel milk. Renc. Rech. Ruminants, 12: P. 399.

[23] Guiraud J.P. (2003). Food Microbiology. Ed Dunod, paris : p 91-92-292.

[24] Allouche F.N, Hellal A, and Laraba A, (2010). Etude de l'activité antimicrobienne des souches de lactobacilles thermophiles utilisées dans l'industrie laitière. Review « nature and technology » n°03.

[25] Belkeziz L, (2020). Lactobacilli: physiology role and interests in human health, university Mohammed V, Rabat P: 26-27.

[26] Sharpe M. E., Fryer T. F. and Smith D. G. (1966): Identification of the lactic acid bacteria. In Gibbs B. M., Skinner F. A. Ed. Identification methods for microbiologist's. Academic Press, London.

[27] Guiraud J. P. (1998). Microbiology of main food proucts, food Microbiology. 1e Ed , Dunod. Paris.

[28] Hariri A., Ouis N., Sahnouni F., and Bouhadi D., (2009). Mise en œuvre de la fermentation de certains ferments lactiques dans des milieux a base des extraits de caroube. Review. Microbiology. Ind. P: 37-55.

[29] Simmons, J.S. (1926). A culture medium for differenciating organisms of typhoid-colon oerogenes groups and for isolation of certain fungi. J. Infect. Dis., 1926, 39 : 209.

[30] Kandler, O., and Weiss, N. (1986).Genus LactobacillusBeijerinck 1901, 212AL. In: Sneath, P.H. A., Mair, N. S., Sharpe, M. E., and Holt, J. G. (Eds). Bergey's manual of systematic bacteriology (8nd ed.). Baltimor. pp 208-1234.

[31] Koort J, Vandamme P, Schillinger U, Holzapfel W.H, and Bjorkroth J, (2004). *lactobacillus curvatus subsp.* Melibiosus a later synonym of *lactobacillus sakei subsp*. Carnosus. Int. J. Syst. Evol. Microbiol., 54 p. 1621-1626.

[32] Conter M, Muscariello T, Zanardi E, Ghidini S, Vergara A, Campanini G, and Ianeri A, (2005). Characterization of lactic acid bacteria isoled from an Italian dry fermented sausage. Ann. Fac Medic.Vet Parma 25:167-174.

[33] Priyanka, S. et Prakash, A. (2009). Screening of Lactic Acid Bacteria for Antimicrobial Properties Against Listeria monocytogenes Isolated from Milk Products at Agra Region. Internet Journal of Food Safety, 11:81-87

[34] Gänzle, M.G., (2015). Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Current Opinion in Food Science 2, 106–117.

[35] Klein G, Pack A, Bonaparte C, and Reuter G. (1998). Taxonomy and phyziology of probiotic lactic acid bacteria. International Journal of food Microbiology vol 41: 103-125.

[36] Stiles M.E and Holzapfel W.H , (1997). Lactic acid bacteria of foods and their current taxonomy. Int. J. Food. Microbiol 36, 1-29.

[37] Mathialagan M, Edward Y.J.T, David P, Senthilkumar M, Srinivasan M, and Mohankumar S, (2018). Isolation, Characterization and Identification of Probiotic Lactic Acid Bacteria (LAB) from Honey Bees. *International journal of current microbiology and applied sciences*, 7 ; 894-906.

[38] Asfour H.A., Gamal I.M., and Darwish S.F (2016). Phenotypic characterization and molecular identification of some lactic acid producing bacteria in raw milk of different animal species.*Assiut Veterinary Medical Journal*, 62 47-59.

[39] Gaspar P, Carvalho A. L., Vinga S., Santos H. and Neves A. R. (2013). From physiology to systems metabolic engineering for the production of biochemicals by lactic acid bacteria. Biotechnology advances, 31**,** 764-788.

[40] Pringsulaka O., Thogngam N., Suwannasai N., Atthakor W., Pothivejkul K., and Rangsiruji A., (2011). Partial characterization of bacteriocin produced by lactic acid Regulation In: Drider, D., and Rebuffat, S. (Eds). Prokaryotic Antimicrobial Peptides: From Genes toApplications. Springer Verlag. Jaen, Spain. pp 253-390.

[41] Ao X, Zhang X, Shi L, Zhao K , Yu Y, Dong L, Cao Y, and Cai Y (2012). Identification of lactic acid bacteria in traditional fermented yak milk and evaluation of their application in fermented milk products. *Journal of dairy science*, 95 1073-1084.

[42] De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, and Whitmanet WB (2009). Genus *Lactobacillus, Bacillus* and *Listeria.* In: « Bergey's manual of systematic bacteriology - The Firmicutes » Vol 3. Springer éd., New York. pp.19-511.

[43] Coeuret, V, Dubernet, S, Bernardeau, M, Gueguen, M. and Vernoux, J. P. (2003). Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Le Lait, 83, 269-306.

[44] Bennani, S., Mehiouer, K., Rokni, Y., Meziane, M. (2017). Characterization and Identification of Lactic acid bacteria isolated from Moroccan raw cow's milk. Journal of Materials and Environmental Sciences. Vol 8(S):4934-4944.

[45] Zhang S, Zhang L, Jiao Y, Luo X, Li H, Xin L, Xue C, Zhang Y, Yi H, and Han X, (2014) Technological characterization of lactic acid bacteria protease isolated from traditional chinese fermented milk. Journal of Food Quality, 37; 395-402.

[46] Thompson J.K, Collins M.A, and Mercer W.D (1994). Characterisation of a proteinaceous antimicrobial produced by Lactobacillus helveticus CNRZ 450. J. Appl. Bacteriol, 80: 338,348.

[47] Carr, F. J., Chill, D. and Maida, N. (2002). The lactic acid bacteria: A literature survey Critical reviews in microbiology, Rev. Microbiol. 28: 281-370.

[48] Badis A, Guetarni D, Boudjema B.M., Henni D, and Kihal M, (2004). Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. *Food Microbiology*, 21 ; 579-588.

[49] Yelnetty A, Purnomo H, Purwadi, Mirah A. (2014). Biochemical characterization of lactic acid bacteria with proteolytic activity and capability as starter culture isolated from spontaneous fermented local goat milk. Journal of Natural Sciences Research.4.

[50] Pelmont Jean (1993). Bactéries et environnement. Adaptations physiologies. Presses universitaire de Grenoble.

[51] Axelsson L. (2004). Classification and physiology. In: Lactic acid bacteria: Microbiological and functional aspects (Salminen S, Wright AV et Ouwehand A). 3e Ed : Marcel Dekker, Inc. NewYork. 1-66.

[52] Holzapfel W.H, Haberer P, Geisen R, Björkroth J. and Schillinger U (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. Am. J.Clin. Nutr, 73: 365S–73S.

[53] König H, and Fröhlich J. (2017). "Lactic Acid Bacteria," in Biology of Microorganisms on Grapes, in Must and in Wine. Springer International Publishing, p. 3–41.

[54] De Almeida Júnior W.L.G, Da Silva Ferrari Í, De Souza J.V, Da Silva C.D.A, Da Costa M.M, and Dias F.S, (2015). Characterization and evaluation of lactic acid bacteria isolated from goat milk. Food control, 53 ; 96-103.

[55] Ceapa C, Lambert J, Limp KV, Wels M, Smokvina T, Knol J, Kleerebezem, (2015). Correlation of Lactpbacillus rhamnosus genotypes and carbohydrate utilization signatures determined by phenotype profiling. Appl Environ Microbiol 81(16): 5458-5470.

[56] Ozgan D, and Vural HC (2011). Identification of *Lactobacillus* strains isolated from faecal specimens of babies and human milk colostrum by API 50 CHL system. J.M.G.G.3 (3):46-49.

[57] Bernardoa M.P, Coelhoa, L.F, Sassa D.C, Contieroa J. (2016). Lactic acid production by Lactobacillus rhamnosus B103 from dairy industry waste. Braz. J. Microbiol.

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