PJN
ISSN 1680-5194
ansinet.com/pjn

PAKISTAN JOURNAL OF NUTRITION

Science Alert
scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2021.77.82

Research Article
Assessment of Lactic Acid Bacteria Isolated from White Cheese (Gibna Bayda) Produced in El Dueim City, White Nile State-Sudan

Asmahan Azhari Ali Mohammed

Department of Food Science and Human Nutrition, Faculty of Agriculture and Veterinary Medicine, Qassim University, Buraiydah, P.O. Box: 6640-Buraidah: 51452, Kingdom of Saudi Arabia

Abstract

Background and Objective: Lactic acid bacteria (LAB) have been used for centuries as food preservative in food technology and food production. The objective of the study was to isolate and identify naturally occurring lactic acid bacteria from Gibna Bayda produced in local markets in El-Dueium City, Sudan. **Materials and Methods:** A total of 25 white cheese (Gibna Bayda) samples were collected from individual local cheese producers in El Dueim City in White Nile state. Microbiological analysis of the cheese samples was performed. **Results:** Among 92 LAB strains isolated from Gibna Bayda, bacilli were accounted for 70 isolates (76.09%), while cocci were 22 isolates (23.91%). The isolated LABs were dominated by *Lactobacillus bulgaricus* (22.8%), followed by *L. fermentum* (18.48%), *Streptococcus lactis* (17.4%) and *L-casei* (13.04%). The least isolated LABs were *L. helveticus* (3.26%). **Conclusion:** Dominant LAB (*Lactobacilli* and *Streptococci*) were successfully isolated from Gibna Bayda. The wide diversity in microbial community could be attributed to variations in the specific environmental conditions found in Sudan as well as the manufacturing processes for indigenous Gibna Bayda.

Key words: White cheese, fermented milk, Gibna Bayda, lactic acid bacteria, dairy products

Citation: Asmahan Azhari Ali Mohammed, 2021. Assessment of lactic acid bacteria isolated from white cheese (Gibna Bayda) produced in El Dueim City, White Nile State–Sudan. Pak. J. Nutr., 20: 77-82.

Corresponding Author: Asmahan Azhari Ali Mohammed, Department of Food Science and Human Nutrition, Faculty of Agriculture and Veterinary Medicine, Qassim University, Buraiydah, P.O. Box: 6640-Buraidah: 51452, Kingdom of Saudi Arabia Tel/Fax: (016)3800882

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gibna Bayda produced at different sites of many cities in White Nile states like El Dueim and Rabak[1]. The highest production of Gibna Bayda is obtained during the rainy season[2]. Gibna Bayda is a traditional fermented milk containing high concentrations of salt (Sodium Chloride) which is added to the milk before processing. Also the processing includes heating of the fresh milk at 35°C followed by addition of salt to give 6-10% solution in milk.

Traditional dairy products differ from commercial dairy products in taste, flavour and the consistency due to the natural flora of the lactic acid bacteria present in it[3]. Cheese is a very involved microbial ecosystem and a very complex microflora develops in most cheese varieties. The microflora plays a major role in cheese ripening and selection of suitable strains would enable the cheese maker to control or modify flavor development. However, due to the complexity of the flora and the interactions which occur between individual components of it and the cheese environment, strain selection for flavor improvement is not always very obvious[4]. Lactic acid bacteria (LAB) are naturally present in milk and milk products. Many LAB species play an important role in the ripening process of cheese, especially to improve the consistency, aroma and flavor. Certain LAB strains are characterized by their ability to transform lactose and improves the digestibility of fermented dairy products[5] as well as their preservation[6]. They also employed for improvement of the taste, texture and viscosity in the manufacture of dairy products[7]. Lactic acid bacteria can be recovered from fermented foods and beverages, vegetables, milk and milk products.

This is in addition to its ability to withstand cheese manufacturing processes (whether fresh or ripe), it can grow in a moisture content of 39% and a salinity between 4 and 6% of sodium chloride (NaCl) and the pH ranges from 9.4-3.5.

Today, lactic acid bacteria (LAB) are a focus of intensive international research for their essential role in most fermented food. Lactic acid bacteria have the ability to produce various antimicrobial compounds promoting probiotic properties[8] including anti-tumoral activity[9,10]. They can reduce serum cholesterol[11,12], alleviate lactose intolerance[13], stimulate the immune system[14] and stabilize gut microflora[15]. The objective of the study was to isolate and identify naturally occurring lactic acid bacteria from Gibna Bayda produced in local markets in El-Dueim City, Sudan.

MATERIALS AND METHODS

Sample collection: The study was conducted from February to April 2020 to isolate and identify lactic acid bacteria in Gibna Bayda (Fig. 1). A total of Twenty-Five Gibna samples were collected from different local markets in the city of El Dueim, White Nile State, Sudan. Samples were kept in a refrigerator (around 4°C) until the analysis was started.

Isolation of lactic acid bacteria: A total of 10 gm of each item (Gibna Sample) was obtained from five different local markets in El Dueium city, aseptically and transferred to the separate sterile container, containing 90 mL of sterile saline solution. The latter was shaken well until a homogeneous dispersion of 1:10 dilution obtained, then serially diluted and inoculated on plates[16], where *Lactococci* were grown on M17 agar (Oxoid) and enumerated after 48h of incubation at 32°C[17]. *Lactobacilli* were grown on MRSA; Merck, carried out anaerobically using the gas pack system at 30°C for 48h[18].

Identification of the bacterial strains: Morphological and Biochemical characteristics were considered for identification of lactic acid bacteria genera. Preserved isolated strains were tested for gram staining, catalase production and spore formation using the method of Harrigan and McCance[19]. The other tests were: anaerobic growth[20], Motility test[21], oxidase test[22] and fermentation/oxidation test[23]. Hydrolysis of arginine, citrate utilizations, gas formations from glucose in MRS broths containing inverted Durham tubes, dextran productions from sucrose in MRS+ST agar, growth on different temperature (10, 37 and 45°C) for 5 days, resistance to 60°C for 30 min (Sherman test), growth in the presence of 4 and 6.5% (w/v) NaCl and different pH (4.5 and 6.5) and changes in turbidity of MRS broth after 24, 48 and 72 h of incubations were implicated to identify the strains[24-26]. Arginine MRS medium and Nessler reagent were employed to perform the hydrolysis tests as described by Yavuzdurmaz[27]. Citrate utilization and colored colonies growth were observed in SL and D agars

Fig. 1: Gibna Bayda

and results were interpreted according to Reddy *et al*.[28] and Kempler and McKay[29]. Sugar fermentation tests Membrane (0.45 µm) filtered 1% (w/v) solutions of different sugars (glucose, fructose, lactose, galactose, maltose and mannitol) were deployed to study fermentation characteristics of the isolates. Nutrient broth (0.8%) with 1 mL phenol red was autoclaved at 121+1°C for 15 min then cooled to room temperature. Five ml of broth and 100 µL of sugars were taken into sterilized test tubes. These tubes were checked for contamination by placing at room temperature for 24 h. After 24 h, the purified colonies were inoculated into test tubes with specific sugar containing broth and incubated at 37°C for 48 h. The positive test for sugar fermentation was indicated by color change from red to yellow in the test tubes as mentioned by Mehmood *et al*.[30].

RESULTS AND DISCUSSION

LAB strains isolated, purified and further identified and differential tests were applied including morphological and physiological characteristics which facilitate the opportunity for identification of the LAB. The identification results were confirmed by the carbohydrates fermentation and assimilation profile obtained in correlation with bergey's manual and also using the manuals of Sharpe[25], Holt *et al*.[31], Garrity *et al*.[32] and Hardie[33].

The isolated LABs were dominated by the species *Lactobacillus bulgaricus* (22.8%), followed by *L. fermentum* (18.48%), *Streptococcus lactis* (17.4%) and *L-casei* (13.04%). The least isolates were *L. helveticus* (3.26%) (Table 1).

Table 2 shows the general properties of the 92 LAB strains isolated from Gibna Bayda. Among these LAB isolates, bacilli were accounted for 70 isolates (76.09%), while cocci were 22 isolates (23.91%). The isolates were classified into two genera according to De Vos *et al*.[34]:

- ***Streptococci*:** All of coccal shaped isolates were Gram's stain positive, catalase negative, grow at 10°C and at 45°C, didn't grow in 4% (w/v) NaCl and produce lactic acid without production of gas (CO_2). All isolates in this genus ferment glucose, lactose and fructose and produce lactic acid. They can't produce acid from maltose, mannitol, melezitose, raffinose, rhamnose, sucrose. So, 6 isolates were considered to be *Streptococcus thermophilus* and 16 isolates were considered to be *Streptococcus lactis*
- ***Lactobacilli*:** Seventy rod shaped isolates were also identified as *Lactobacillus*. They were non motile, gram's stain positive, catalase negative, grow at 10°C and at 45°C, unable to grow in 4% (w/v) NaCl and they produce lactic acid without gas (CO_2). The bacilli shape isolates were subdivided into six species:

  - ***Lactobacillus bulgaricus*:** Twenty-one of the rods shaped isolates was capable to produce acid from Glucose, lactose, Galactose, mannitol, maltose, Ribose and trehalose but not from Raffinose, Xylose, melezitose, sucrose and rhamnose
  - Twelve of these isolates were capable to produce acid from glucose, lactose, fructose, maltose, mannitol, melezitose, sucrose but not from raffinose and xylose and sorbitol. These 12 isolates were identified as *Lactobacillus casei*
  - ***Lactobacillus helveticus*:** These are 3 isolates which were able to produce acid from glucose and lactose and unable to produce acid from fructose, maltose, mannitol, melezitose, raffinose, rhamnose and cellobiose
  - ***Lactobacillus brevis*:** These are 8 isolates which produced acid from raffinose and sorbitol and not from maltose and melezitose
  - ***Lactobacillus fermentum:*** These isolates produced acid from arabinose and sucrose
  - ***Lactobacillus plantarum*:** These are 9 isolates which were able to produce acid from glucose, Melibiose and Arabinose but not from raffinose and xylose

Table 1: Identification of lactic acid bacteria isolated from Gibna Bayda cheese

Sample	No. of Isolates	Isolates	No.	Percentage
Gebna Bayda samples	92 isolates for all samples	*Streptococcus lactis*	16	17.40
		Lactobacillus bulgaricus	21	22.80
		L-plantarum	9	9.78
		L-casei	12	13.04
		L. fermentum	17	18.48
		L. helveticus	3	3.26
		S. thermophilus	6	6.52
		L. brevis	8	8.70

Table 2: Biochemical tests for identification of Lactic acid genera from Gibna Bayda cheese

Gram stain	+	+	+	+	+	+	+	+
Catalase activity	-	-	-	-	-	-	-	-
Morphology	Cocci	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Cocci	Bacilli
Production of CO_2 from glucose	-	-	-	-	+	-	-	-
Growth at temperature								
10°C	+	+	+	+	-	+	+	-
37°C	+	+	+	+	+	+	+	
45°C	-	+	+	+	+	+	+	+
Growth at								
6.5% NaCl	-	-	+	+	-	-	-	+
4% NaCl	+	-	-	-	+	-	-	+
Arginine hydrolysis	+	+	+	+	-			-
Esculin hydrolysis	+	+	+	+	+	+	+	-
Dextran production	+	+	+	+	-	-	-	-
Citrate utilization	+	+	-	+	+	-	-	+
Sugar fermentation tests								-
Glucose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	-
Galactose	+	+	+	+	+	-	-	+
Sorbitol	-	+	-	-	-	-	-	+
Melibiose	-	-	+	+	+/-	-	--	+
Raffinose	-	-	-	-	-	-	+	+
Xylose	+	-	-	-	-	--	-	+
Sucrose	+	+	+	+	+	-	-	-/+
Arabinose	-	+	+	+	+	-	-	-/+
Melezitose	-	-	-	-	-	+/-	-	-
Rhamnose	-	+/-	-	-	-/+	-	-	+
Maltose	+	+	+	+	-	-	-	-
Ribose	+	+	+	+	+	-	-	-/+
Mannitol	+	+	+	+	+	-	-	-/+
Trehalose	+	+	+	+	+	-	+	+
Cellobiose	+	+	+	+	+	-	+	-/+
Identified as	*S. lactis* (16) isolates	*L. bulgaricus* (21) isolates	*L. casei* (12) isolates	*L. plantarum* (9) isolates	*L. fermentum* (17) isolates	*L. helveticus* (3) isolates	*S. thermophilus* (6) isolates	*L. brevis* (8) *isolates*

+: positive reaction, -: negative reaction, -/+: variable reaction

Lactobacilli commonly found in the gastrointestinal tract of animals and humans can also be found in fermented food such as milk and milk product. The creamy or whitish appearance of the isolated *Lactobacillus* species on MRS agar confirms that *Lactobacilli* have dominance in fermented milk products when compared to other lactic bacteria[35].

Lactic acid bacteria (LAB) are widely used as starters in fermented dairy products, mainly *Streptococcus thermophilus*, *Lactococcus lactis*, *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, in which the proteolytic system plays a vital role[36]. The non-starter LAB (NSLAB) are very important in cheese ripening, such as *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus curvatus*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Pediococcus acidilactici*, *Pediococcus pentosaceus* and other LAB[37]. During the process of milk fermentation, LAB hydrolyze milk proteins, thereby releasing bioactive peptides. The characteristics of this process are important to scale up the fermentation at industrial levels[38,39].

These findings agree with some previous studies on the isolation of *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* from dahi of Himalayas[40] and *L. lactis* subsp. *cremoris* from Kazerun's traditional fermented yoghurt[41].

CONCLUSION

This study showed a clear picture of microbial diversity and density in Sudanese white cheese (Gibna Bayda) that might largely contribute to its typical texture and flavor. Dominant LAB comprising of *Lactobacilli* and *Streptococci* were successfully isolated from Gibna Bayda which is locally produced in El Dueim City (Sudan). The wide diversity in microbial community could be attributed to variations in the specific environmental conditions found in Sudan as well as the manufacturing processes for indigenous Gibna Bayda. Selection, propagation and preservation of the best performing strain to make starter culture could be done in future through genetic characterization.

REFERENCES

1. El-Tayeb, G.D., 1986. The Cheese Industry and Under Development in the White Nile. United Nations University press, Tokyo, pp: 150.
2. Hamid, O.I.A. and O.A.O. El Owni, 2007. Microbiological properties and sensory characteristics of white cheese (*Gibna bayda*) collected in Zalengei area West Darfur. Res. J. Anim. Vet. Sci., 2: 61-65.
3. Kieronczyk, A., S. Skeie, T. Langsrud and M. Yvon, 2003. Cooperation between *Lactococcus lactis* and non-starter lactobacilli in the formation of cheese aroma from amino acids. Applied Environ. Microbiol., 61: 734-739.
4. Beresford, T.P., N.A. Fitzsimons, N.L. Brennan and T.M. Cogan, 2001. Recent advances in cheese microbiology. Int. Dairy J., 11: 259-274.
5. Weinberg, Z.G., O. Shatz, Y. Chen, E. Yosef, M. Nikbahat, D. Ben-Ghedalia and J. Miron, 2007. Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silages. J. Dairy Sci., 90: 4754-4762.
6. Abdelbasset, M. and K. Djamila, 2008. Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk Raib. Afr. J. Biotechnol., 7: 2908-2914.
7. Soukoulis, C., P. Panagiotidis, R. Koureli and C. Tzia, 2007. Industrial yogurt manufacture: Monitoring of fermentation process and improvement of final product quality. J. Dairy Sci., 90: 2641-2654.
8. Temmerman, R., B. Pot, G. Huys and J. Swings, 2002. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. Int. J. Food Microbiol., 81: 1-10.
9. De Vuyst, L. and B. Degeest, 1999. Heteropolysaccharides from lactic acid bacteria. FEMS Microbiol. Rev., 23: 153-177.
10. Ostlie, H.M., H.M. Helland and J.A. Narvhus, 2003. Growth and metabolism of selected strains of probiotic bacteria in milk. Int. J. Food Microbiol., 87: 17-27.
11. Desmazeaud, M., 1996. Les bactéries lactiques dans l'alimentation humaine : Utilisation et innocuité. Cahiers Agric., 5: 331-343.
12. Jackson, M.S., A.R. Bird and A.L. McOrist, 2002. Comparison of two selective media for the detection and enumeration of *Lactobacilli* in human faeces. J. Microbiol. Methods, 51: 313-321.
13. De Vrese, M., A. Stegelmann, B. Richter, S. Fenselau, C. Laue and J. Schrezenmeir, 2001. Probiotics—compensation for lactase insufficiency. Am. J. Clin. Nutr., 73: 421s-429s.
14. Isolauri, E., Y. Sütas, P. Kankaanpää, H. Arvilommi and S. Salminen, 2001. Probiotics: effects on immunity. Am. J. Clin. Nutr., 73: 444s-450s.
15. Gibson, G.R., J.M. Saavendra, S. MacFarlane and G.T. MacFarlane, 1997. Probiotics and Intestinal Infections. In: Probiotics 2, Application and Practical Aspects, Fuller, R. (Ed.). Chapman and Hall, London, UK., pp: 10-39.
16. Salfinger, Y. and M.L. Tortorello, 2015. Compendium of Methods for the Microbiological Examination of Foods. 5th Edn., American Public Health Association Washington, D.C., 1.
17. Terzaghi, B.E. and W.E. Sandine, 1975. Improved medium for lactic streptococci and their bacteriophages. Applied Microbiol., 29: 807-813.

18. De Man, J.C., M. Rogosa and M.E. Sharpe, 1960. A medium for the cultivation of lactobacilli. J. Applied Bacteriol., 23: 130-135.
19. Harrigan, W.F. and M.E. McCance, 1976. Laboratory Methods in Food and Dairy Microbiology. 1st Edn., Academic Press, London, pp: 25-29.
20. Kiss, I., 1984. Testing Methods in Food Microbiology. Elsevier Science, Amsterdam, Pages: 447.
21. Barrow, G.I. and R.K. Feltham, 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3nd Edn., Cambridge University Press, England, ISBN: 9780521543286, Pages: 331.
22. Steel, K.J., 1961. The oxidase reaction as a taxonomic tool. J. Gen. Microbiol., 25: 297-306.
23. Hugh, R. and E. Leifson, 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol., 66: 24-26.
24. Mayeux, J.V., W.W.E. Sandine and P.R. Elliker, 1962. A selective medium for detecting *Leuconostoc* organisms in mixed strain starter cultures. J. Dairy Sci., 45: 655-656.
25. Sharpe, M.E., 1979. Identification of the Lactic Acid Bacteria. In: Identification Methods for Microbiologists, Skinner, F.A. and D.W. Lovelock (Eds.). Academic Press, London, pp: 233-259.
26. Samelis, J., F. Maurogenakis and J. Metaxopoulos, 1994. Characterisation of lactic acid bacteria isolated from naturally fermented Greek dry salami. Int. J. Food Microbiol., 23: 179-196.
27. Şebnem, H.H. and H. Yavuzdurmaz, 2007. Isolation, characterization, determination of probiotic properties of lactic acid bacteria from human milk. M.Sc. Thesis, Izmir Institute of Technology
28. Reddy, M.S., E.R. Vedamuthu, C.J. Washam and G.W. Reinbold, 1969. Differential agar medium for separating *Streptococcus lactis* and *Streptococcus cremoris*. Applied Microbiol., 18: 755-759.
29. Kempler, G.M. and L.L. McKay, 1980. Improved medium for detection of citrate-fermenting *Streptococcus lactis* subsp. *diacetylactis*. J. Applied Environ. Microbiol., 39: 926-927.
30. Mehmood, T., T. Masud, S.A. Abbass and S. Maqsud, 2009. Isolation and identification of wild strains of lactic acid bacteria for yoghurt preparation from indigenous dahi. Pak. J. Nutr., 8: 866-871.
31. Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Lippincott Williams and Wilkins, Baltimore, USA., ISBN-13: 9780683006032, Pages: 787.
32. Garrity, G.M., J.A. Bell and T.G. Lilburn, 2004. Taxonomic Outline of the Prokaryotes. Bergey's Manual of Systematic Bacteriology. 2nd Edn., The Williams and Wilkins Co., Baltimore, Md USA.
33. Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt, 1986. Bergey's Manual of Systematic Bacteriology. 1st Edn., Williams and Wilkins, Baltimore, USA., pp: 1104-1139.
34. Vos, P., G. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.-H. Schleifer and W. Whitman, 2009. Bergey`s Manual of Systematic Bacteriology. Vol. 3. Springer-Verlag, New York, Pages: 1450.
35. Oliveira, M.N., I. Sodini, F. Remeuf and G. Corrieu, 2001. Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. Int. Dairy J., 11: 935-942.
36. Savijoki, K., H. Ingmer and P. Varmanen, 2006. Proteolytic systems of lactic acid bacteria. Applied Microbiol. Biotechnol., 71: 394-406.
37. Settanni, L. and G. Moschetti, 2010. Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiol., 27: 691-697.
38. Korhonen, H., 2009. Milk-derived bioactive peptides: From science to applications. J. Funct. Food, 1: 177-187.
39. Tzvetkova, I., M. Dalgalarrondo, S. Danova, I. Iliev, I. Ivanova, J.M. Chobert and T. Haertle, 2007. Hydrolysis of major dairy proteins by lactic acid bacteria from Bulgarian yogurts. J. Food Biochem., 31: 680-702.
40. Dewan, S. and J.P. Tamang, 2007. Dominant lactic acid bacteria and their technological properties isolated from the himalayan ethnic fermented milk products. Antonie van Leeuwenhoek, 92: 343-352.
41. Azadnia, P., M. Shah Ahmad Ghasemi, M. Davanian Mohaghegh, M. Karimi Jashni, M.H. Zamani, A. Khalegh Babaki and N. Taarof, 2011. Isolation and identification of Lactococci from traditional yoghurt in tribes of Kazerun J. Anim. Vet. Adv., 10: 698-700.