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The Use of Lactic Acid Bacteria (LAB) Isolates as Single and Mixed-Strain Starter Culture in Yoghurt Processing

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ABSTRACT

Lactic acid bacteria (LAB) were isolated from cowmilk yoghurt by culturing the milk on specific media and pure culture was obtained by sub-culturing. Preliminary identification of lactic acid bacteria was made on the basis of Gram-staining and catalase reaction followed by microscopic examination to observe cell arrangement and morphological characteristics. After incubation, individual colonies were selected and moved into sterile brooths (MRS or M17 medium for enrichment). The enriched bacteria were purified using streak plate technique. Gram positive and catalase negative cocci and bacilli colonies were stored in glycerol solution as lactic acid bacteria. Cowmilk yoghurt contained lactic acid producing bacteria, four bacterial strains were isolated, according to biochemical identification and were characterize as: *Lactobacillus rhaminosus-2*, *Lactobacillus fermentum*, *Lactobacillus paracasei-2*, and *Lactobacillus licheniformis-2*. The isolates were characterized on the basis of their morphological, biochemical, physiological and16S rRNA gene sequences. All of the isolates were well identified with the help of molecular techniques. Cowmilk yoghurt was prepared by using one of these isolates as starter culture as single or mixed strain. Treatment A which is the combination of the commercialized starter culture (yogourmet) with one of the LAB isolates (Lactobacillus fermentus) had better attributes for use as starter culture as its results met with yoghurt recommended standards. It is concluded that yoghurt can be prepared successfully from LAB isolates and better results can be obtained by coagulating milk with commercialized starter culture.

KEYWORDS

Yoghurt; Lactic acid bacteria; Biochemical identification; Morphological; Starter culture

INTRODUCTION

Micro-organisms are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the Lactic Acid Bacteria (LAB) which is used in making starter culture for dairy products. The proper selection and balance for starter culture is critical for the manufacture of fermented products of desirable texture and flavour. The microbiological quality of milk and milk

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products is influenced by the initial flora of raw milk [1]. Lactic acid bacteria (LAB) are widely used in fermented food production and are considered as generally recognized as safe (GRAS) organisms which is safely applied in medical and veterinary functions [2].

Members of LAB share the property of being Gram-positive bacteria that ferment carbohydrates into energy and lactic acid [3]. In addition, LAB produces small organic compounds that give the aroma and flavor to the fermented product [4]. In the food industry, LAB is widely employed as starter cultures and has been indexed as part of human microbiota [5]. It has also been recognized that LAB are capable of producing inhibitory substances other than organic acids (lactate and acetate) that are antagonistic toward other microorganisms [6]. LAB is used commonly in milk product which is very important in human development and health [7,8].

Fermented foods are popularly accepted for their flavour, better keeping quality, and the fact that fermentation creates variety among foods. Yoghurt is a cultured milk product that is produced through a fermentation process. [9] defined yoghurt as a fermented milk product that evolved empirically some centuries ago by allowing naturally contaminated milk to sour at a warm temperature, in the range of 40°C - 50°C. The micro-organisms which are used conventionally in this process are referred to as "starter culture". They include *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* Traditionally, different bacteria have been involved in the fermentation of milk but according to the Codex Alimentarius definition [10], the coagulated, fermented milk product can only be called "yoghurt'' if the bacteria synergically grown in the milk are *Streptococcus thermophilus* (new nomenclature: *Streptococcus salivarius ssp. thermophilus*) and *Lactobacillus bulgaricus* (new nomenclature: *Lactobacillus delbrueckii ssp. bulgaricus*). These two species of bacteria are very well-known starter microorganisms of yoghurt fermentation. They grow synergistically in milk, increase the acidity by secreting lactic acid, and coagulate the milk proteins while producing the specific yoghurt aroma compounds. In some countries, there is a legal requirement for *Lactobacillus delbrueckii ssp. bulgaricus* to be included in the starter culture of any dairy product labeled as 'yoghurt', because its typical flavour of yoghurt depends on the presence of *Lactobacillus delbrueckii ssp bulgaricus*. In some countries, less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii ssp. lactis*, are sometimes mixed with the starter culture [11].

Yoghurts come in a variety of textures (liquid, set and stirred curd), fat contents (regular fat, low-fat and fat-free) and flavours (natural, fruit, cereal and chocolate) and can be consumed as a snack or part of a meal, as a sweet or savory food. This versatility, concomitant with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population subgroups [11]. As the popularity of yoghurt products continues to grow, manufacturers are continuously investigating value-added ingredients to entice health-conscious consumers [12]. The main biochemical changes that occur in cheese manufacture is the production of lactic acid from lactose. This is achieved by different species of lactic acid bacteria (LAB). The responsible flora that forms acid development during cheese production are starter cultures that cause decrease in the pH, formation of curd, expulsion of whey [13]. In order to improve the spontaneous traditional fermentation, [14] suggested controlled fermentation using mesophilic lactic acid bacteria starter culture. Isolation of lactic acid bacteria (LAB) from fruits and vegetables have frequently been reported [15,16].

In order to develop and test the potency of the isolated LAB isolates (*Lactobacillus fermentus* and *Lactobacillus licheniformis*) a suitable starter for controlled fermentation, isolation and identification of the dominant bacteria involved in the fermentation of milk products and the use of isolates as starter cultures is essential. Therefore, the objective of this study was to use the isolated isolates

(*Lactobacillus fermentus* and *Lactobacillus licheniformis*) as starter culture as single and mixed strain starter culture in yoghurt manufacture.

MATERIALS AND METHOD

Raw Material Sourcing and Preparation

Sample collection

The fresh cowmilk was obtained from Teaching and Research Unit, Dairy Farm, University of Ibadan, while the commercial freezed-dried yoghurt starter culture (yogourmet) was purchased from Oojo market, Ibadan.

Cowmilk yoghurt preparation

Fresh cow milk obtained was sieved, in other to remove any foreign matter like hair and dirts during milking; pasteurized at 73°C for 20 minutes (with the use of water bath), cooled to 43°C. The yoghurt culture (*Lactobacillus bulgaricus, Streptococcus thermophillus* and *Lactobacillus accidophillus*) 5 grams was mixed to a litre of milk pasteurised and incubated at 43°C for 11 hours. Thus, yoghurt was allowed to cool to 4°C to form cow milk yoghurt [17].

Identification, Isolation and Molecular Characterization of Lactic Acid Bacteria from the Yoghurt Sample

Pour plate technique

Lactic acid bacteria were isolated from cowmilk yoghurts using de Man, Rogosa and Sharpe (CM0361 Oxoid, England) according to the method of [18].

Biochemical Identification

Gram staining procedure

According to the method of [18].

Catalase test

According to the method of [18].

Growth at different temperatures

at 15°C and 45°C are the most frequently used for the classification of *bacilli* [19].

Inoculation and Isolation of Bacteria

MRS (de Man, Rogosa and Sharpe) agar was used for the isolation of lactic acid bacteria. Serial dilutions up to 10^{-7} were prepared using pour plate technique. Plates were incubated anaerobically in an anaerobic jar (BBL, GasPak) at 32°C for 48 hours. Bacterial colonies showing clear zones were selected, streaked twice on MRS agar plates for purification and maintained as pure culture over nutrient agar slants (pH 9.0, 4°C). The individual bacterial colonies were stored in 0,8% MRS agar overlaid with glycerol at -20°C. The isolates having clearance zone were selected for colony morphology and some physiological tests. Physiological and biochemical identifications were performed according to the methods and criteria of [20].

Molecular Characterization

DNA isolation

Genomic DNA from all isolates was extracted by using [21].

Separation of amplification products and purification of PCR products

The presence of DNA fragments with the size of 1500 bp - 2000 bp indicated that the amplification was achieved.

Sequencing the DNA

The sequence data obtained was deposited to NCBI database with BLAST analysis for molecular identification of the organisms.

Using LAB Isolates as Single and Mixed-Strain Starter Cultures in Yoghurt Production

Sample preparation and method

The cowmilk prepared was measured and divided into two equal parts, for experimental treatments:

A = commercial freezed-dried yoghurt starter culture (yogourmet) and Lactobacillus fermentus isolate (1:1).

B = Lactobacillus fermentus isolate and Lactobacillus licheniformis isolate (1:1).

Two separate freshly prepared yoghurts were incubated at 43°C for 11 hours and stored at 4°C for further analysis. Different physical and chemical analysis like ash, fats, total solids and moisture and protein analysis were carried out on the yoghurt samples. Alkalinity and acidity were determined using phenolphthalein as marker by titration of 0.1 N NaOH while pH was determined by using pH meter (Melter Delta 340) after standardization at pH of 4.0 and 7.0.

RESULTS AND DISCUSSION

The results obtained from the use of LAB isolates as single and mixed-strain starter culture in yoghurt processing are shown in Table 1. Statistical analysis revealed that treatments A and B were significantly different from one another ($p < 0.05$). Treatment A was the combination of the commercialized starter culture (yogourmet) with one of the LAB isolates (*Lactobacillus fermentus*) and Treatment B was combination of two different LAB isolates (*Lactobacillus fermentus* and *Lactobacillus licheniformis*) as starter culture. The average total titratable acidity of treatment A (Starter culture + LAB isolates) was 1.40% while the average total titratable acidity (TTA) of treatment B (LAB isolates) was 1.28%. The average TTA of natural yoghurt was 1.34%. The result of treatment A with respect to findings of [22] in which acidity increased over storage period [23] also observed an increase in TTA during storage period. The pH values of treatments A and B are shown in Table 1. Treatment B had a lower pH value of 3.46, when compared with the pH value 4.12 of treatment A. [24] reported yoghurt's pH as 4.50 as lactic acid bacteria produce lactic acid during fermentation of milk- lactose, thus lowering its pH [25]. Food Standard Code requires that the pH of yoghurt should be a maximum of 4.50 in order to prevent the growth of any pathogenic organism [26]. Gelation and acidification processes of yoghurt are affected by starter culture characteristics. Selection criteria for lactic acid bacteria include acidification rate, aroma, flavour and texture characteristics [27]. Several studies refer to the effect of lactic acid bacteria producing exopolysaccharides on the rheological properties of yoghurt, which are often used to increase the viscosity of yoghurt products [28,29].

The average protein content of treatment A was 3.5 while the average protein content of treatment B was 2.7 and the result for treatment A was in line with the findings of [30] who found that protein contents of low-fat stirred yoghurt ranged from 3.4% to 6.0%. The total soluble sugar (TSS) in treatments A and B were 14.60 brix and 9.40 brix respectively. The decrease in TSS for B may be attributed to yeast utilization of sugars in metabolic processes for energy production. Decreases in total soluble solids in yoghurts from 7.33% to 6.83% and 15.33% to 14.93% for corn milk and cow milk yoghurts respectively, have also been reported and the reductions have been attributed to the utilization of sugar by the starter cultures [31,32].

The average fat contents of treatments A and B which were 0.30% and 0.06% respectively were in line with findings of [30] who reported that fat contents for low-fat stirred yoghurt ranged from 0.3% to 3.5%. Fat content has been reported to have positive influence on the physical and sensory characteristics [33,34] and negative impacts on the shelf stability of yoghurts [35,36]. Using LAB isolates as single or mixed strain starter culture, treatment A had better attributes for use as starter culture as its results met with yoghurt recommended standards.

Treatments	Total Titratable Acidity (%lactic acid)	pH	Moisture Content (%)	Protein Content (%)	Fat Content (%)	Total Content Solids (^{0}brix)
A	1.40^a	4.12^a	87.65^b	3.5^a	0.30^a	14.6^a
B	1.34^b	3.46^b	91.74^a	2.7^b	0.06^b	9.4^b

Table 1: Means of physical and chemical analysis of single and mixed strain starter cultures.

Means in a column with the same letter are not significantly different ($p < 0.05$).

Treatment A (Starter culture + LAB isolates).

Treatment B (LAB isolates + LAB isolates).

CONCLUSION

Based on the finding of the present study, it is concluded that yoghurt can be prepared successfully from the use of isolated LAB (*Lactobacillus fermentus*) in combination with commercialized starter culture (yogourmet) and better results was obtained. Treatment A was a better selection for starter culture as its results met with yoghurt recommended standards.

The yoghurt making technology can also help in freeze drying the LABs to improve its potency, their economic conditions by reduced cost and by finding a suitable market for the LAB.

DECLARATION OF INTEREST

The authors declare no conflict of interest whatsoever before, during or after this study.

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REFERENCES

1. Ritcher RL, Vadamuthu R (2001) Microbiology Examination of Food. 4th (Edn.), American Public Health Association: 483-485.
2. Holzapfel WH, Haberer P, Geisen R, et al. (2001) Taxonomy and important features of probiotic microorganisms in food and nutrition. The American Journal of Clinical Nutrition 73(2): 365s-373s.
3. Jay JM (2000) Modern Food Microbiology 6th (Edn.), Aspen Publishers Inc., Maryland, US: 121-391.
4. Caplice E, Fitzgerald GF (1999) Food fermentations: Role of microorganisms in food production and preservation. International Journal of Food Microbiology 50(1-2): 131-149.
5. Sanders ME (2000) Symposium: Probiotic bacteria: Implications for human health: Considerations for use of probiotic bacteria to modulate human health. Journal of Nutrition 130: 384S-390S.
6. Daeschel MA (1989) Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technology 43: 164-167.
7. Tannock GW, Tilsala-Timisjarvi A, Rodtong SNGJ, et al. (1999) Identification of *Lactobacillus* isolates from the gastrointestinal tract, silage, and yoghurt by 16S-23S rRNA gene intergenic spacer region sequence comparisons. Applied and Environmental Microbiology 65(9): 4264-4267.
8. Shinoda T, Kusuda D, Ishida Y, et al. (2001) Survival of *Lactobacillus helveticus* strain CP53 in the human gastrointestinal tract. Letters in Applied Microbiology 32(2): 108-113.
9. Ihekoronye AI, Ngoddy PO (1985) Integrated Food Science and Technology for the Tropics, London, Macmillan Publishes: 343.
10. FAO (1992) Codex standard for fermented milks- CODEX STAN 243-2003. Rome: FAO/OMS.
11. Mckinley MC (2005) The nutrition and health benefits of yoghurt. International Journal of Dairy Technology 58(1): 1-12.
12. Allgeyer LC, Miller MJ, Lee SY (2010) Drivers of liking for yogurt drinks with prebiotics and probiotics. Journal of Food Science 75(4): S212-S219.
13. Beresford TP, Fitzsimons NA, Brennan NL, et al. (2001) Recent advances in cheese microbiology. International Dairy Journal 11(4-7): 259-274.
14. Abu-Tarboush HM (1996) Comparison of associative growth and proteolytic activity of yogurt starters in whole milk from camels and cows. Journal of Dairy Science 79(3): 366-371.

15. Duangjitcharoen Y, Kantachote D, Ongsakul M, et al. (2008) Selection of probiotic lactic acid bacteria isolated from fermented plant beverages. Pakistan journal of biological sciences: Pakistan Journal Biological Science 11(4): 652-655.
16. Abiona SO, Adegoke GO (2017) Molecular characteristics of probiotics lactic acid bacteria isolated from soursop, cowmilk, goatmilk yoghurts and cheese. Journal of Food Biotechnology Research 1(1): 1-7.
17. Dirar HA (1993) The indigenous fermented foods of the Sudan: A study in African food and nutrition. CAB international, Wallingford, UK: 303-344.
18. Dave RI, Shah NP (1996) Evaluation of media for selective enumeration of *Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus,* and *bifidobacteria*. Journal of Dairy Science 79(9): 1529-1536.
19. Yvon M, Rijnen L (2001) Cheese flavour formation by amino acid catabolism. International Dairy Journal 11(4-7): 185-201.
20. Bulut C, Gunes H, Okuklu B, et al. (2005) Homofermentative lactic acid bacteria of a traditional cheese, Comlek peyniri from Cappadocia region. Journal of Dairy Research 72(1): 19-24.
21. Thottappilly G, Thresh JM, Calvert LA, et al. (2003) Cassava: In virus and virus-like diseases of major crops in developing countries. Loebenstein G, Thottappilly G (Eds.), Kluwer Academic Publishers: 800.
22. Tarakçi Z, Kucukoner E (2003) Physical, chemical, microbiological and sensory characteristics of some fruit-flavored yoghurt. Van Veterinary Journal 14(2): 10-14.
23. Guler A, Mutlu B (2005) The effects of different incubation temperatures on the acetaldehyde content and viable bacteria counts of bio-yoghurt made from ewe's milk. International Journal Dairy Technology 58: 174-179.
24. Varnam A, Sutherland JP (2001) Milk and milk products: Technology, chemistry and microbiology. Springer Science & Business Media.
25. Eke MO, Olaitan NI, Sule HI (2013) Nutritional evaluation of yoghurt-like product from baobab (Adansonia digitata) fruit pulp emulsion and the micronutrient content of baobab leaves. Advance Journal of Food Science and Technology 5(10): 1266-1270.
26. Donkor ON, Henriksson A, Vasiljevic T, et al. (2006) Effect of acidification on the activity of probiotics in yoghurt during cold storage. International Dairy Journal 16(10): 1181-1189.
27. Bouzar F, Cerning J, Desmazeaud M (1997) Exopolysaccharide production and texture-promoting abilities of mixed-strain starter cultures in yogurt production. Journal of Dairy Science 80(10): 2310-2317.
28. Duboc P, Mollet B (2001) Applications of exopolysaccharides in the dairy industry. International Dairy Journal 11(9): 759-768.
29. Laws AP, Marshall VM (2001) The relevance of exopolysaccharides to the rheological properties in milk fermented with ropy strains of lactic acid bacteria. International Dairy Journal 11(9): 709-721.
30. Janhoj T, Petersen CB, FroSt MB, et al. (2006) Sensory and rheological characterization of low-fat stirred yogurt. Journal of Texture Studies 37(3): 276-299.
31. Vasiljevic T, Jelen P (2002) Lactose hydrolysis in milk as affected by neutralizers used for the preparation of crude β-galactosidase extracts from Lactobacillus bulgaricus 11842. Innovative Food Science & Emerging Technologies 3(2): 175-184.
32. Wang YC, Yu RC, Chou CC (2002) Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. Food Microbiology 19(5): 501-508.

33. Bille PG and Keya EL (2002) A comparison of some properties of Vat-heated and dry skim milk powder fortified set yoghurts. Journal of Food Technology in Africa 7(1): 21-23.
34. Marinescu A, Pop F (2009) Variation in physicochemical parameters of probiotic yoghurt during refrigeration storage. Carpathian Journal of Food Science and Technology 1(2): 18-26.
35. Saint-Eve A, Lévy C, Le Moigne M, et al. (2008) Quality changes in yogurt during storage in different packaging materials. Food Chemistry 110(2): 285-293.
36. Farinde EO, Adesetan TO, Obatolu VA, et al. (2009) Chemical and microbial properties of yogurt processed from cow's milk and soymilk. Journal of Food Processing and Preservation 33(2): 245-254.