



Studies on the Effectiveness of Wild and Commercial strains of Lactic Acid Bacteria Starter Culture during Backsloping for Yoghurt Production

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Yogurt is one of the traditionally fermented dairy products which are usually prepared with lactic acid bacteria cultures containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. This starter culture could be prepared in addition to other bacteria as probiotics. Yoghurt has gained widespread consumer acceptance as a healthy diet. Yoghurts products are produced in Nigeria by using the imported starter culture bacteria and has played significant roles in helping dairy farmers convert their short-life, highly perishable fresh milk to fairly more stable products like cheese and yoghurt which are highly nutritious too for both low-and high-income earners. However, there are concerns over the growing food and food-related import dependency in the sub-African countries and Nigeria in particular which have increased during the last few years. The selection of indigenous starter culture strains with the capacity for backsloping in yoghurt production might reduce importation and improve economy in a way. A fundamental benefit of backsloping is that it reduces the fermentation time while successfully maintaining sensory attributes of the product. The goal of this study was to determine the effects of backsloping fermentation of milk using indigenous and

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commercial (Exotic) strains of Lactic Acid Bacteria. Yoghurt fermentation was carried out by backsloping method using three commercial (Exotic) formulated cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; ES07, ES32 and ES14 respectively as well as wild (isolated) formulated culture strains; WS11, WS65 and WS13 respectively and were used to produce yoghurt using backsloping method. The overall sensory attributes of yoghurts produced using exotic compared with indigenous strains showed no significant ($p > 0.05$) difference in terms of acidification and coagulation ability. The pH of fermented products using commercial cultures ranged from 4.53-5.32, While pH of yoghurts produced using isolated cultures ranged from 4.35 – 4.72, total plate count 2.7×10^7 - 2.3×10^8 cfu/ml during the 10 hour fermentation times. The overall acceptability were in the decreasing order; WS65 > WS11 > ES07 > WS13 > ES14 > ES32. From this order, backsloping has equal advantages when both local and commercial starter cultures are used. Therefore, Starter culture formulation using indigenous strains could be further developed genetically for commercial applications because they compete favourably with their exotic counterparts. Hence, the current challenges of importation due to Covid-19 economic reality and foreign exchange will reduce to a minimal if the indigenous strains are utilised.

Keywords: Acidification; backsloping; cultures; fermentation; indigenous LAB; organoleptic and yoghurt.

1. INTRODUCTION

There is high demand for yoghurt and fermented milk owing to their numerous health benefits. In recent years, more people are getting aware of the health protections that could be derived from the consumption of controlled-fermented products such as yoghurt. [1]. High consumption of yogurt is often attributed to higher opportunity to contribute to a healthy lifestyle because the product provides excellent source of highly bioavailable protein and calcium as well as a source of probiotics that may provide different levels of health benefits (Shahnawaz *et al.*, 2013). Yogurt has also been ranked as a very valuable and popular fermented dairy products consumed worldwide with great consumer acceptability and high health benefits such as provision of milk proteins, essential amino acids for good health, as well as improvement of lactose tolerance, immune enhancement, metabolic disorder and prevention of gastrointestinal disorders [2].

Fermented products like yoghurt have been known to contain elements with some therapeutic and health maintenance properties [3]. Aside the above mentioned benefits, the presence of *S. thermophilus* and *L. bulgaricus* as the culture organisms in the fermented products like yogurt means the availability of functional probiotic properties [4]. The use of Lactic Acid Bacteria (LAB) strains in dairy starter cultures is highly recommended because they are Generally Regarded As Safe (GRAS status) [5].

Some studies have shown that another beneficial property of LAB is their possession of

biomolecules with autophagy triggering capacity which are said to be important in cell recycling for antimicrobial protection and maintenance of epithelial competence [6]. Although LAB is wide spread in nature, they are commonly associated with dairy materials where they mostly play the role of acid fermentation. These functions have been harnessed for the commercial production of many dairy products with good sensory characteristics for the benefit of mankind [7]. Indigenous Lactic acid bacteria strain isolated from the spontaneous fermented milk are referred to as the 'wild' have specific properties that can be harnessed for controlled fermentation of milk [7].

Considering the limited availability of exotic commercial starter cultures in Nigeria, there has been a trend for the selection and development of new starter cultures from the autochthonous habitat for development into viable commercial status with potent traits for backsloping applications and stable shelf life. The advantages of using such newly-isolated and well-characterized cultures for use in place of the exotic strains will lead to the production of standard products with predictable characteristics as their exotic peers in terms of both nutrition enhancement and health promoting features.

There is currently limited documentation of the technological consequences of the different traditional techniques employing backsloping for producing controlled fermented products such as yoghurt.

Therefore, the aim of this work was to study some backslipping methods of yoghurt production using commercial strains and those of the isolated cultures. As such, indigenous knowledge in traditional fermentation practices utilising backslipping method may enhance further development, up-scaling and utilization of local resources to the level of other renowned fermented milk products.

2. MATERIALS AND METHODS

2.1 Study Area

For the isolation and characterisation of starter cultures from the wild, 25 *nono* samples were bought from some homemade processors and local markets in Kuje and Lugbe, FCT, Abuja, Nigeria. The samples were transported in a cooler to the National Biotechnology Development Agency laboratory, Abuja, Nigeria.

2.2 Sample Collection

Commercial starter cultures for this research were obtained from *Shoprite* Supermarket, Airport Road, Lugbe, Abuja, Nigeria and were kept at 4°C in a refrigerator until they were needed for analysis- [8].

2.3 Isolation of Lactic Acid Bacteria

One (1ml) ml aliquot was taken from the collected samples aseptically after thorough homogenization. Serial dilution was then made using sterile peptone water by adding 1ml into 9ml. A 0.1ml aliquots 10^{-2} and 10^{-4} of the serially diluted samples were aseptically added to M17 and MRS agar for the isolation of LAB (Badis *et al.*, 2004a). In order to prevent the growth of yeast, the agar media were supplemented with 100mg^{-1} of cycloheximide and were incubated for 5 days at 40°C anaerobically using the Gas Pack system (Merck Anaerocult type A) [9]. After incubation, discrete colonies were randomly selected and purified on sterile media. The resulting pure strains were kept at 4°C for MRS and M17 plates and at -20°C for M17 and MRS broths supplemented with 20% glycerol for further use [10]. [8].

2.4 Identification of the Bacterial Strains

All the purified bacteria strains were tested for gram reaction, catalase production and spore formation. Colonies were characterized on MRS and M 17 agar. Only strains with gram positive and catalase negative reactions were finally used

for further identification [11]. Growth at different temperatures (10, 15, 37, 40 and 45°C) for 5 days, resistance to 63°C for 30 min (Sherman test), growth in the presence of 2, 3, 4 and 6.5% NaCl and different pH (4.5 and 6.5) were used for further identification of the strains. Ability to hydrolyse arginine and asculin, utilization of citrate, production of acetone, gas formation from glucose and dextran production from sucrose were also tested according to the method of Philip *et al.*, 2017.—The strains were also tested for fermentation of L-arabinose, D-xylose, galactose, D-fructose, sorbitol, lactose, melibiose, saccharose, D-raffinose, melezitose, mannose and glucose. The growth of bacterial strains at 10, 15, 37, 40 and 45°C was visually confirmed by the changes in turbidity of MRS or M17 broth after 24, 48 and 72 hours —of incubation. The tolerance of microorganisms to the different levels of salt, pH and heat (60°C) was also visually evaluated. Arginine dihydrolase agar and asculinazid agar (Merck, Germany) were employed to perform the hydrolysis tests. For evaluation of citrate utilization and acetone production, citrate and MR-VP agars (Merck, Germany) were used. MRS or M17 broths containing inverted Durham tubes were used for evaluation of gas production and the production of dextran from sucrose was done in MRS agar [12].

2.5 Preservation of LAB Strains

In order to prevent loss of properties, the identified strains were stored in Skimmed milk with 30% (v/v) glycerol at 4°C till further use [12]. Cultures were also kept on MRS agar or M17 agar slant at 4°C and streaked forth-nightly.

2.6 Technological Characterization

2.6.1 Acidifying activity

The acid-production potential of the strains was measured according to the method of Julijana *et al.*, 2016. Fermentation was carried out at 40°C by preparing 10% milk and introducing 24 hour old cultures at 1% rate. The pH was measured during the 12 hour fermentation period by inserting the cleaned probe of the pH meter in the fermenting medium and determining the values

2.6.2 Proteolytic activity

The proteolytic activity of the isolates were determined by fermenting 10% skimmed milk for 12 hours at 40°C. Coagulation was recorded as either positive (+) or Negative (-).

2.6.3 Biomass production

The bacteria strains were sub-cultured into MRS broth (100ml) of the medium with 10% of the active culture. The respective growth was monitored by measuring the Optical Density at 600nm (OD600) using the Vernier SpectroVIS Spectrophotometer. The biomass was determined by centrifuging aliquots (1ml) sample. The dry weight was determined by drying the pellet of samples at 55°C for 24h [13].

2.7 Experimental Design for Yoghurt Production

In order to fully attain the goal of this research, two sets of milk fermentation experiments were set-up in parallel; one used commercial starter culture strains, while the second one used co-culture samples of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (ES07, ES32 and ES14) respectively while the other one used the isolated and purified LAB strains, (WS11, WS65 and WS13) respectively.

The freeze dried samples were prepared from the commercial strains by weighing 0.5g of the lyophilized cultures and thereafter was added to 5ml of pasteurised milk. The culture was incubated at 40°C for 18 hours. Three sets of reconstituted skimmed milk (10%) were homogenised and pasteurized at 65°C for 30 minutes and cooled to 42°C. Each set was inoculated with about 5ml (containing 10^6 CFU/mL) commercial, prepared starter cultures (ES07, ES32 and ES14). Five (5ml) of isolated cultures (WS11, WS65 and WS13) containing 10^6 CFU/ml which were determined by the initial viable counts. All the set-up was incubated at 40°C until the pH reached 4.5 then kept in the refrigerators set at 4°C. Sample from the fermented samples were used to initiate consecutive batch (2.5%) to get a three-fold sample. During fermentation, the pH, coagulation, aerobic total plate count, flavour and organoleptic properties were measured for each set of the sample. The third-fold fermented sample was compared.

2.8 Organoleptic Analysis

This analysis was carried out by ten taste panellists who were trained to identify the characteristics of yoghurt without sugar added. They were served with the chilled samples in coded cups and the respective records were made after tasting. The panellists were asked to rank the coded samples on the basis of their quality attributes such as consistency, flavour, taste, colour, texture and overall acceptance using nine-point hedonic scale, expressing degree of liking or disliking as in the questionnaire [3].

3. RESULTS

Table 1 shows the pH, Proteolytic ability and total plate count of both the commercial and isolated LAB strains recorded at the end of a third-fold milk fermentation (Backsloping). The lowest pH (4.35) was recorded in the yoghurt culture fermented with isolated lactic acid bacteria culture (WS65) which is the most favourable pH for milk coagulation and acid-protection of the fermented product expected for yoghurt to be formed and preserved. This made isolated strain to have performed better than commercial sample. Mean while, the highest pH (5.32) was recorded in yoghurt fermented with bacteria culture (ES32). This makes Total aerobic plate count of yoghurt culture were recorded overall higher in yoghurt cultures fermented with isolated lactic acid bacteria strains than in the commercial strains. The highest being 2.3×10^8 CFU/mL (WS11) while the lowest was 1.1×10^6 CFU/mL (ES14). Shortening of lag phase during fermentation processes might be responsible for higher increase in total plate count and lower pH values that were recorded which is a better yoghurt quality.

Fig. 1 shows the mean percentage acceptance of the yoghurts produced by the various lactic acid bacteria cultures. The highest being WS65 (84%) while the lowest was ES32 (50%).

Table 1. pH, proteolytic ability and total plate count of the third-fold fermented yoghurt using commercial and Isolated LAB strains

Parameter	Commercial cultures			Isolated cultures		
	ES07	ES32	ES14	WS11	WS65	WS13
pH (EPV)	4.53	5.32	5.21	4.42	4.35	4.72
Proteolysis	+	+	+	++	++	+
Total Plate Count	4.0×10^7	3.4×10^6	1.1×10^6	2.3×10^8	1.2×10^8	2.7×10^7

EPV = End point value; += Coagulation

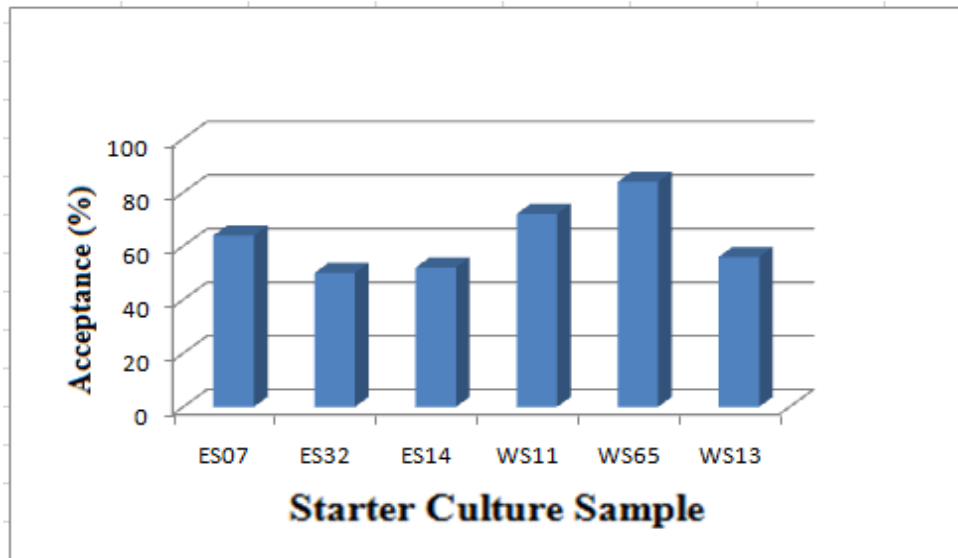


Fig. 1. The percentages acceptance of the yoghurt samples produced by the respective cultures



A. Soft, smooth, pudding-like, firm textured product- consistent throughout back sloping batches



B. Smooth-and-creamy Product

C. Panel of Taste Analysts

Fig. 2. Comparative back sloping ability of some lactic acid bacteria strains

4. DISCUSSION

The average acidification activity of the isolated lactic acid bacteria culture was better than their commercial counterparts. This might be due to the shortening of lag phase of the microorganisms responsible for fermentation processes as a result of adaptation during backsloping. Bacteria culture with shortened lag phase has cells that are able to synthesize enzymes and factors needed for cell division and population growth under their new environmental conditions. The population then enters the log phase, in which cell numbers increase [14]. The process also enhanced the overall qualities of the yoghurt product as shown by the panelists' result. The principle of backsloping have been used successfully for *pito* and *khisk* (Local fermented products) production respectively means that the practice could be applied in large scale yoghurt processes [15]. The practice could be useful for enhancing industrial processes because cultures are optimally utilised which are basis for industrial expansion for fermented foods.

Also, the high acid production means that the product could be better preserved thereby giving it longer shelf life because acid content of food matrix reduce the population spoilage microorganisms. It is said that within a spoiling food, there is often a succession of different populations that rise and fall as different nutrients become available or are exhausted. Notably, some microbes, such as lactic acid bacteria, secrete compounds such as acid that inhibit competitors [16-22]. The aerobic total plate count recorded higher values in products fermented with indigenous strains. The implication is that such strains could be better as probiotic cultures since they would be delivered into the gastro-intestinal tract in larger concentrations resulting in better and more colonization for enhanced activity. According to the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO), (2014), probiotics are defined as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host.

5. CONCLUSION

From the data and observations recorded from our study, it can be concluded that backsloping method could be used for large-scale production of yoghurt because there was no significant difference in the performance of the backsloped

lactic acid bacteria cultures as compared with the commercial strains. In fact, the yoghurts produced with indigenous strains showed better overall acceptance over the ones produced with commercial strains.

6. RECOMMENDATIONS

This old traditional method of fermentation should be employed/upscaled for large scale yoghurt production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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