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ISOLATION OF LACTIC ACID BACTERIA (LAB) FOR PRODUCTION OF YOGHURT FROM LOCAL GOAT AND COW MILK

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Lactic acid bacteria (LAB), yoghurt, starter culture, Goat-milk, cow-milk, herders' camps

ABSTRACT

Industrial organisms are sought everywhere and they could be priceless in their applications. In this study 15 Lactic Acid Bacteria (LABs) were isolated from two animal breeds (goat milk and cow milk) sampled from 11 villages and two markets site in Bida metropolis using de Mann Ragossa and Sharpe (MRS) agar, these isolates were of five genera according to their morphological and biochemical characteristics, they were *Lactobacillus fermentum* (20.0%), *Lactobacillus* sp. (33.3%), *Leuconostocs* sp. (6.67%), *Pediococcus* sp. (33.3%) and *Streptococcus* sp. (6.67%). They were screened for their yoghurt production tendencies and the yoghurt produced was assessed by a panel of sensory analysts. All of the LAB isolated from cow and goat milk samples produced relativelygood yoghurt and the quality of the product can be improved with further research on the right combination of the culture to create a multicultural starter. It is recommended local cow and goat milk around Bida metropolis will serve as a natural sources of good starter culture for production of yoghurt.

INTRODUCTION

The starter is a critical ingredient in yoghurt manufacture. Yoghurt is a dairy product produced by bacteria fermentation of milk sugar (lactose) into lactic acid. This gives yoghurt its gel-like texture and characteristics taste. Yoghurt is a fermented milk product and is one of the famous fermented milk preparations. The word yoghurt is derived from the Turkish word "jugurt" which means dense thick [1]. It is the most widely available fermented milk in western world where its popularity derives more from its flavour and versatility [1] It is often sold with flavour such as vanilla, strawberry and chocolate but can be without any flavour. Some are even sweetened. Yoghurt nutritionally good and many of the have protective or therapeutic functions have been known for hundreds of years [1]. These beneficial attributes have lesser attention in Africa. Yoghurt is a food produced by bacterial fermentation of milk [2]. Yoghurts are made from preserved bacterial culture known as yoghurt cultures which are often made up of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* but other lactic acid bacteria are also utilized.

The fermentation of lactose by these bacteria is able to produce lactic acid, which acts on milk protein to give yoghurt its texture and characteristic tart flavour [2]. Yoghurt consists of water, fat, protein, sugar and minerals (ash), hence could be helpful in enhancing the microflora of the gut. It can be produced from different milk such as goat, cow, sheep, horse, water buffaloes, skimmed milk, non-fat milk or low-fat milk including milk from plant origin such as soymilk. Lactic acid bacteria are known in the food industries, and mostly used organisms for making yoghurt. They are positive to Grams reaction, rod to cocci shaped, acid tolerant, do not produce spore but have the ability to produce lactic acid. One of the bacteria for making yoghurt is *Lactobacillus bulgaricus*, which can grow at 45°C, but some strains cannot survive longer time in yoghurt which reduces the organoleptic characteristics and probiotic effect [3]. However, LAB such as *L. amylovorous, L. helveticus, L. amylophiius, L. casei, L. brevis and L. plantarum* could also be used for yoghurt production [4]. Yoghurt is one of the major sources of vitamins and minerals, it contains higher vitamins (vitamins B12) than fresh milk [5]. the growing interest in the development of a variety of fermented milk products for beneficial purposes are due to the beneficial effects of fermented milk products are as a result of the presence of a variety of bioactive compound produced by lactic acid bacteria (LAB). Starter culture are purchased from foreign countries and production of yoghurt have become more expensive due to the foreign exchange and devaluation of Nigerian currencies. There is also the need to verify the sources of starter cultures due to the proliferation of Genetically Modified Organism (GMO) many of which might not meet the criteria got Generally Regarded As Safe (GRAS) and the attendant disadvantages. To this end, the production of yoghurt from LAB isolated from local goat and cow milk becomes important.

AIM AND OBJECTIVES

The primary aim of this research work is to evaluate the production of yoghurt from lactic acid bacteria isolated from local goat and cow milk and their potential for the production of good quality yoghurt that compares with other popular standard products.

JUSTIFICATION OF THE STUDY

The starter culture is a critical ingredient in yoghurt manufacture, commercial starter culture for production of yoghurt are expensive and, in many cases, need special storage which often require electricity and eventually make yoghurt production difficult in Nigeria. The rate of acid production by yoghurt culture should be synchronized with plant production schedule. For LAB to be used for yoghurt it needs to be a bio-protective starter cultures, they must possess a range of physical and biochemical characteristics, they must be able to grow rapidly and produce sufficient antimicrobial metabolites, which must be demonstrated in the specific food environment [6].

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Thirteen different samples of raw milk from cow and goat were aseptically collected from herders' camp, villages around Bida, more samples were purchased from raw milk vendors in New Market and Small market in Bida, Niger State. The samples were aseptically taken in ice pack to the microbiology laboratory of Federal Polytechnic Bida for microbiological analyses and assessment.

ISOLATION AND CHARACTERIZATION OF ISOLATES

Lactic acid bacteria (LAB) were enumerated and isolated from samples of raw milk from local goat and cow, using pour plate and streaking techniques respectively. and phenotypically identified by reference to Bergey's Manual of Systematic Bacteriology and an Approach to the Classification of Lactobacilli [2]. The pure colonies which are of different size, shape, and colour may be isolated or transferred into test tubes containing liquid culture media (broth) or directly inoculated on the solid agar media by streak plate method for making pure cultures [7] and [8].

DETERMINATION OF LACTIC ACID PRODUCTION

One loopful of 24 hrs old culture of the LAB isolates containing 10^6 CFU/mL were inoculated into 20 mL of MRS broth, and incubated at 24, 48, 72 and 96 hrs. The production of lactic acid was determined by titrating 20 mL of MRS broth containing LAB isolates at different incubation periods of 24, 48, 72, and 96 hrs with 0.1M of NaOH and l mL of phenolphthalein indicator (0.5% in 50% alcohol). The titratable acidity was calculated as lactic acid (% v/v). The quantity (ml) of the lactic acid in the yoghurt that neutralises 20ml of IN NaOH that was determined in triplicate. The lactic acid was calculated according to [9].

$$Lactic\ Acid\ content = \frac{\text{V1 NaOH x N NaOH x M.E.x100}}{Volume\ of\ sample\ x\ 100} =$$

Where NaOH = Volume of NaOH used, N NaOH = Normality of NaOH solution M.E. = Equivalence Factor

DETERMINATION OF DIACETYL PRODUCTION

Diacetyl production capacity of the isolates was determined by separately transferring 25 mL of MRS broth containing one loopful of 24 hrs old culture of the LAB isolates at different incubation time of 24, 48, 72, and 96 hrs into 100 mL of conical flasks. The flasks were titrated against 0.IN HCl using bromophenol blue as indicator [9] with a greenish yellow colour indicative of the end point. Hydroxylamine was used for residual titration.

$$\text{Ak} = \frac{(b-s)(100E)}{W}$$

Where; K = Percentage of diacetyl, B = No of mL of 0.IN HCl used in titration, E = Equivalence factor, W = Volume of sample, S = No of mL of 0.IN HCl consumed in titration of samples.

PRODUCTION OF YOGHURT WITH SELECTED STARTER CULTURES

Yoghurt samples were prepared using a slightly modified method of Rahmann *et al*. (1999). The inoculum size (10^6 CFU/mL) of the selected LAB starter cultures were obtained using Mcfarland standard 0.5. 100ml of raw milk was measured into sterile glass bottles and were pasteurized at 65°C for 30 minutes with the use of a water-bath it was rapidly cooled to 35°C. The pasteurized milk samples were inoculated with 1.0 mL of selected starter cultures containing inoculums size of 10^6 CFU/mL. using this as bulk starters at 4% inoculum level for an incubation period of 2.5 to 3.5 hours at 42°C. The production of flavour by yoghurt cultures is a function of time as well as the sugar content of yoghurt mix. After inoculation, the contents were thoroughly mixed, and incubated at 42°C for 4-6 hrs using a thermostatically controlled water-bath, and rapidly cooled to 4°C. However, the yoghurt samples were stored at 4°C (cold storage). Yoghurt produced was compared with a commercial yoghurt was used as positive control.

pH OF YOGHURT PRODUCED USING STARTER CULTURE

Yoghurt produced from milk using different LAB isolates as starters, yoghurt produced with commercial starter culture and commercial yoghurt were tested for pH using pH meter [9]

ORGANOLEPTIC ANALYSES

Yoghurt samples were randomly numbered and a panel of 15 judges that are familiar with the consumption of yoghurt and are conversant with such properties as taste, colour, odour and flavour, their consents were sought to evaluate a day and 2 weeks old starter yoghurt for flavour, body-texture, appearance and overall acceptability. A 7-point hedonic scale of 1 (dislike extremely) and 7 (like extremely) was used for the sensory evaluation.

STATISTICAL ANALYSIS

The experiments carried out in triplicates. Data was analysed using descriptive statistics and One-way Analysis of Variance (ANOVA) with Duncan Multiple Range Test to discriminate differences between means with significance at P≤0.05 according to statistical procedure (Statistical Analysis Systems (2018) SPSS Version 20 IBM). Means and standard deviation will also be presented in tables.

RESULT AND DISCUSSION

Enumeration of the Lactic acid bacterial population in both cow milk and goat milk collected from the thirteen different points of production and sales around Bida was carried out. The lactic acid bacterial count of cow and goat milk (Table 1) was determined and it was observed that the lactic acid bacteria (LAB) count in goat milk from the village inside the Federal Polytechnic, Bida was highest (4.55 X 10^7 ±15.0) and it was higher than the count on cow milk from the same village while village 8 has the lowest LAB count (2.73 X 10^2 ±1.38) on goat milk.

The LAB count for cow milk was highest (4.48 X 10^8 ±1.68) for milk procured from the vending point in Bida New Market, while the LAB count on cow milk was least for village 4 (2.61 X 10^2 ±2.33) and there is significant statistical difference (p>0.05) between the two samples. The high count on cow milk from the Bida New market could be because of the time lag between milking and the time of sales which have allowed for the multiplication in the bacterial and resultant increase in population. Both raw cow and goat milk are rich sources of lactic acid bacteria.

TABLE 1: LACTIC ACID BACTERIAL COUNT OF MILK FROM COW AND GOAT produced and SOLD IN MARKET AROUND

BIDA METROPOLIS.

Location	Mean CFU count + SD	
	Goat milk ± SD	Cow milk ± SD
Small Market	$1.33 \times 10^7 \pm 2.55^a$	$8.64 \times 10^7 \pm 0.23^a$
New Market	$1.64 \times 10^7 \pm 2.78^a$	$4.48 \times 10^8 \pm 1.68^b$
village 1	$4.55 \times 10^7 \pm 15.0^b$	$1.61 \times 10^6 \pm 3.37^a$
village 2	$3.32 \times 10^3 \pm 1.17^a$	$6.62 \times 10^2 \pm 3.17^a$
village 3	$2.36 \times 10^3 \pm 0.57^a$	$6.12 \times 10^2 \pm 2.19^a$
village 4	$5.88 \times 10^2 \pm 2.53^a$	$2.61 \times 10^2 \pm 2.33^a$
village 5	$1.43 \times 10^3 \pm 1.11^a$	$1.30 \times 10^3 \pm 1.13^a$
village 6	$3.78 \times 10^3 \pm 1.12^a$	$2.94 \times 10^3 \pm 1.87^a$
village 7	$5.35 \times 10^3 \pm 1.56^a$	$5.97 \times 10^2 \pm 3.78^a$
village 8	$2.73 \times 10^2 \pm 1.38^a$	$4.88 \times 10^2 \pm 2.33^a$
village 9	$4.62 \times 10^2 \pm 0.75^a$	$7.42 \times 10^2 \pm 4.14^a$
village 10	$6.10 \times 10^2 \pm 3.25^a$	$1.45 \times 10^3 \pm 2.52^a$
village 11	$8.54 \times 10^2 \pm 4.07^a$	$9.81 \times 10^2 \pm 8.33^a$

Note: the result is a mean of three determinations. Different superscripts within a column indicate significance ($p<0.05$)

SENSORY EVALUATION OF YOGHURT PRODUCED USING LAB ISOLATE AS STARTER CULTURE

The result of the sensory evaluation of yoghurt prepared with 5 isolate of lactic acid bacteria (LAB) from goat and cow milk as compared on (7-hedonic scale) to a branded yoghurt (Hollandia) sold in Nigeria. With (7=extremely good, 6=very good, 5=Good, 4=Slightly good, 3=Bad, 2=Very bad and 1=extremely bad). The results are as shown on Table 2.

The appearance of yoghurt produced, LAB1 gave the poorest appearance while that of LAB6 is the best from the isolate but the appearance is significantly different ($p<0.05$) from that of the control the appearance of the control (Hollandia) yoghurt probably because it was subjected to industrial processing under a more controlled environment.

The odour of yoghurt prepared with the 5 organisms isolated (CMRS1, GMRS1, GMRS2, GMRS3 and LAB 5) from cow and goat milk gave an odour that is a significantly different from the control. GMRS2 gave the poorest odour described as unfriendly, does not smell good while GMRS4 gave the best odour.

In terms of colour presentation, yoghurt prepared with GMRS1 gave the poorest colour presentation as assessed by the 15-men sensory panel, there is significant difference in the colour of yoghurt prepared with the isolate when compared to the colour of the branded yoghurt (Hollandia). This could also be as a result of additional controlled colour added to the industrially produced yoghurt as part of the quality control to create uniform product.

The taste of yoghurt prepared with the five lactic acid bacteria isolates (CMRS1, GMRS1, GMRS2, GMRS3 and CMRS2) from cow milk and goat milk. There is no significant difference in taste among the five lactic acid bacteria isolates and the taste is significantly different from that of branded yoghurt. The acceptance in taste of the control when compared to that of the isolates, the difference could be because the control is sweetened (written on the pack that it is sweetened yoghurt) which confers additional flavor, this have effect on the sensory evaluation of the yoghurt samples.

The pH of the yoghurts produced using different starter culture reflects the effectiveness of the different organism for the acidification of the fresh milk used for production of yoghurt. GMRS2 gave the highest pH (5.23±0.15) while GMRS4 gave the least pH after 24hours. But the pH of CMRS2 was the closest to and not significantly different ($p<0.05$) from the pH of the commercially successful yoghurt (HOLL) used as control.

The level of Lactic was highest in the yoghurt produced using CMRS2 and GMRS3 and least in the yoghurt produced using GMRS1. the lactic acid production capacity of the organisms is not significantly different ($p<0.05$) from the control. This also explains why thr yoghurt from the organisms taste well. The results agree with the findings of Aforijiku et al. (2022), Adesokan et al. (2009).

The yoghurt produced are manageable but when compared with the branded yoghurt the product from the isolates did not match with the control. However, the control was produced using multicultural starter culture while the trial LAB samples in this study were from monoculture starter culture, the control was produced in strictly controlled insdustrial environment and as well sweetened to meet the market expectations.

It is therefore recommended that further studies should be carried out on the combined effect of these isolates, the appropriate combination that may produce yoghurt with higher acceptance and match the most popular yoghurt in Nigeria.

Table 2: Sensory evaluation, pH and acidity of yoghurt produced using LAB isolate as starter culture

ORGANISM	APPEARANCE	ODOUR	COLOUR	TASTE	pH at 24hr	Acidity
CMRS1	2.67 ± 0.62^a	2.87 ± 0.74^{ab}	3.07 ± 0.96^b	1.93 ± 0.80^a	5.20 ± 0.20^c	0.86 ± 0.01^{abc}

GMRS1	2.47±0.83^{a}	3.13±0.74^{bc}	2.33±0.72^{a}	2.00±0.76^{a}	5.13±0.12^{c}	0.82±0.02^{a}
GMRS2	2.80±0.86^{ab}	2.53±0.92^{a}	2.60±0.74^{ab}	2.47±0.83^{a}	5.23±0.15^{c}	0.90±0.04^{bc}
GMRS3	2.87±0.74^{ab}	3.13±0.74^{bc}	3.00±0.76^{b}	2.33±0.82^{a}	4.93±0. 35^{bc}	0.92±0.02^{c}
CMRS2	3.00±0.76^{ab}	2.87±0.74^{ab}	2.73±0.88^{ab}	1.93±0.80^{a}	4.70±0.17^{ab}	0.92±0.01^{c}
GMRS4	3.33±0.90^{b}	3.47±0.74^{c}	2.73±0.59^{ab}	2.00±0.76^{a}	4.57±0.15^{a}	0.85±0.06^{ab}
HOLL	4.60±0.51^{c}	4.73±0.46^{d}	4.80±0.41^{c}	4.67±0.49^{b}	4.62±0.03^{ab}	0.88±0.05^{abc}

Note: the sensory evaluation result is a mean of determinations by 15-member sensory panel, while the pH and acidity is mean of three determinations. Different superscripts within a column indicate significance ($p<0.05$) HOLL is the branded commercial yoghurt

As shown in table 2; there is no statistical difference ($p>0.05$) in the lactic acid bacteria population of milk procured from thirteen different vending points (Bida Small Market, Bida New market and 11 herders camps around Bida). However, the count from herders' camp 1 (Village 1) gave the largest LAB population for goat milk (4.55×10^{7} ±15.0) and is significantly different from the other milk vending points while Village 8 has the least LAB count (2.73×10^{2} ±1.38) from goat milk. New Market was the highest (2.32×10^{8} ± 11.9), while the sample from Small Market gave the lowest LAB count.

Table 3: Biochemical characteristics of isolates

Isolate	Cell morphology	Gram test	Motility	Catalase test	CO_2 from glucose	Fermentation carbohydrate							Growth (% NaCl)			Suspected org.
						Glucose	Lactose	Xylose	Sucrose	Melibiose	Raffin	Sorbitol	2%	4%	6.5%	
CMRS1	Cocci/round	+	-	-	-	-	+	+	+	-	±	±	+	+	-	*Pediococcus* sp
GMRS1	Rods	+	-	-	+	+	+	±	+	±	-	+	-	±	-	*Lactobacillus* sp.
GMRS2	Rods	+	-	-	+	+	+	±	+	±	-	+	-	±	-	*Lactobacillus fermentum*
GMRS3	Cocci/ovoid	+	-	-	+	+	+	±	±	-	-	±	+	-	-	*Leuconostocs* sp.
CMRS2	Rods	+	-	-	+	+	+	±	+	±	-	+	-	±	-	*Lactobacillus* sp.
GMRS4	Cocci/chain	+	-	-	-	+	+	±	±	-	-	-	+	+	-	*Streptococcus* sp.
GMRS5	Rods	+	-	-	+	+	+	±	+	±	-	+	-	±	-	*Lactobacillus fermentum*
GMRS6	Cocci/round	+	-	-	-	-	+	+	+	-	±	±	+	+	-	*Pediococcus* sp
GMRS7	Cocci/round	+	-	-	-	-	+	+	+	-	±	±	+	+	-	*Pediococcus* sp
CMRS3	Rods	+	-	-	+	+	+	±	+	±	-	+	-	±	-	*Lactobacillus fermentum*
CMRS4	Rods	+	-	-	+	+	+	±	+	±	-	+	-	±	-	*Lactobacillus* sp.
CMRS5	Cocci/round	+	-	-	-	-	+	+	+	-	±	±	+	+	-	*Pediococcus* sp
CMRS6	Cocci/round	+	-	-	-	-	+	+	+	-	±	±	+	+	-	*Pediococcus* sp
CMRS7	Rods	+	-	-	+	+	+	±	+	±	-	+	-	±	-	*Lactobacillus* sp.
CMRS8	Rods	+	-	-	+	+	+	±	+	±	-	+	-	±	-	*Lactobacillus* sp.

Selected representative colonies from different dilutions (10% of the observed count) were tested for Gram stain and catalase reaction and further microbiological and biochemical analyses were conducted, as shown on Table 3. And the suspected organisms from the 15 isolates were *Lactobacillus fermentum* (20.0%), *Lactobacillus* sp. (33.3%), *Leuconostocs* sp. (6.67%), *Pediococcus* sp. (33.3%) and *Streptococcus* sp. (6.67%).

These organisms are lactic acid producing bacteria that are capable of carrying out fermentation of milk to produce lactic acid. this means that they may be used as starter culture for the production of yoghurt. This work agrees with [10] and [11] where food items was found to be a good source of LAB bacteria that can improve the preservation of food items. Apart from use in yoghurt production the LAB can also be useful in production of some other industrially important products such as Lactic acid [10] Raw goat and cow milk therefore are good sources of lactic acid bacteria for production of yoghurt as starter culture and preservation of food in Nigeria.

CONCLUSION AND RECOMMENDATION

CONCLUSION

Isolation, characterization and identification of industrially important Lactic Acid Bacteria from goat and cow milk was carried out and the isolates were used for trial production of yoghurt, the 15 isolates were found to comprise of 5 LAB genera and were separately used for production of yoghurt as homofermeters, the yoghurt produced using the isolates were of low quality but not within acceptable range when compare to a commercially successful brand in Nigeria. The result obtained in the study shows that the LAB isolates need to be combined to form a multicultural starter culture which could give a better result. The isolates are not good enough to produce high quality yoghurt. The yoghurt thus produced are however manageable especially in terms of flavor and taste but the colour and odour needed to be improved upon especially when compared with the branded yoghurt.

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