



Screening of Indigenous Bacterial Isolates from Dawadawa (African Locust Beans) in North Central Nigeria

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Abstract: This study was undertaken to screen for Lactic Acid Bacteria (LAB) from indigenous fermented seeds; dawadawa. Isolation of LAB was carried out on MRS Agar. Biochemical, morphological and physiological examinations according to the Bergeys manual were used to identify the bacterial isolates. Six bacterial strains were obtained and given the designations DN, DO, DJ, DT, DG and DH respectively. The obtained colonies were all capable of fermenting lactose, sucrose, trehalose, glucose, maltose and lactulose as the sole carbon source in the broth. Morphological examination revealed that the isolates obtained were non-spore forming gram positive colonies ranging from creamy to white, clear, cocci and rod shaped, smooth textured and flat elevation with transparent opacity. Their growth profile study revealed that all isolates appeared to be mesophilic with progressive growth patterns observed between 24-96 hours at the same parameters for incubation. Isolates DO and DJ were observed to exhibit the fastest growth as determined by measuring optical density of the cells in broth using spectrophotometer at 550nm. Conclusions from the observed experiment indicate that isolated LABs from dawadawa could be applied in other probiotics as starter cultures and be improved for other commercial uses.

Keywords: Lactic Acid Bacteria, Growth Profile, pH, Salinity

1. Introduction

Lactic acid bacteria (LAB) represents a unique group of industrially relevant bacteria, commonly referred to or associated with probiotic bacteria consumed globally in mainly fermented products [1]. This group of gram positive bacteria are easily cultivated from non-milk products including meat, decomposing plant material as well as the gastrointestinal tract whereby they expressly consume and convert these materials, producing lactic acid as the main by-product [2-3]. New frontiers in biotechnology has led to the appreciation of its robust nature that makes it a safe and effective enzymatic powerhouse towards the production of food additives, pharmaceutical compounds and vaccines [4-5]. The industrial drive for harnessing more value-added products from LAB stems from the enhancement of their metabolic properties to the flavour (acetoin, peptides),

texture (exopolysaccharide) and general fortification (riboflavin, folate, cobalamin) of the nutritional value of the food product it is applied to [6-10].

The rich gamut of chemically diverse bioactive compounds obtained from LAB suggests that there is a large collection of LAB in nature for which their existence in our known environments is limited to the region, nature of habitat, temperature, etc. In many African countries, the production of fermentable dairy products does not necessarily include the application of starter cultures, thus suggesting that the fermentation of such products occurs spontaneously from microorganisms emanating from the external environment or the processing equipment [11]. This now advocates for the isolation of a pool of LAB from multiple dairy and non-dairy products towards industrially relevant uses. Factors like the viability of LAB in appreciable quantities within the product is a fundamental requirement for selection.

Data revealed that the daily diet in Africa is primarily fermented food [12], thus making the consumption of dairy an age-old tradition in countries like Nigeria. Like many other African countries, the different tribes in the country consume a range of other fermentable plant products one of which is the African Locust beans (*Parkia biglobosa*) known to the northern part of Nigeria as dawadawa or nère.

The goal of any of such practices would be the preservation of the processed products thereby preventing spoilage as the latter is common [13]. The most prominent LAB species known within this African region within dairy and fermentable non-dairy food products have been identified as *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *dextranicum*, *Lb. curvatus*, *Leuc. Paramesenteroides* and *Lb. plantarum*. In the northern part of Nigeria, dawadawa is the most widely consumed dairy product to the delight of the northern communities [14]. Different strains of lactic acid bacteria are believed to be obtainable from such unique dairy sources [15-16]. The probiotic nature of indigenous dairy products constitutes an integral part of the gastrointestinal flora that boosts host metabolism [17-18]. The aim of this study was to isolate and characterize possible lactic acid bacteria from dairy samples indigenous to northern Nigeria.

2. Materials and Methods

2.1. Isolation of Bacteria

Fresh samples of dawadawa were collected from Gwagwalada Area Council of Abuja. Each was taken in a sterile container separately and placed in a polyethylene bag during transportation to the laboratory employing standard conditions for sample collection. 1g of dawadawa sample (mashed) was weighted aseptically and was suspended in 9ml of 0.15% peptone water under aseptic condition and vortexed for proper homogeneity. 1ml of each thoroughly mixed sample was serially diluted up to 10^{-6} and used to inoculate MRS agar plates supplemented with 100mg/l cycloheximide. Each plate was incubated at 37°C for 48 hours and observed for the growth of colonies. All experiments were carried out in triplicate.

2.2. Identification by Morphological and Biochemical Assessment

Visually distinct colonies were randomly selected and streaked repeatedly on antibiotic supplemented MRS agar to obtain pure isolates. The distinct pure isolates were then subjected to morphological identification with the following parameters: Colour, Shape, Texture, Elevation, Margin, Opacity and gram staining [19]. Wet mounts of isolated cell samples were prepared in distilled water and examined using 40X objective magnification. Biochemical characterization performed included catalase, production of gas from glucose, methyl red-voges proskauer (MR-VP) and carbohydrate fermentation using phenol red broth technique. Sugars used to determine the fermentation profile of the isolates were

fructose, galactose, lactose, maltose, mannitol, arabinose, trehalose, sucrose and lactulose. The cultures were identified based on the pattern of sugar utilization [20].

2.3. Effect of NaCl Concentrations on Growth of Isolates

The isolates were inoculated in MRS broth having different salt concentration (4.5%, 6.5%) and incubated at 37°C for 24–48 hours. The culture tubes were observed for the presence or absence of growth. The development of turbidity in culture tubes was recorded as the ability of isolates to grow at the given salt concentration.

2.4. Effect of pH Concentrations on Growth of Isolates

Pure isolates were inoculated unto MRS broth media with pH values of 4.5 and 9.6 using 1M NaOH and 1M HCl followed by incubation at 37°C for 96 hours. Turbidity was monitored periodically (24 hours) to determine growth measurements were used to determine growth.

2.5. Growth Profile Studies

The isolates were tested for their ability to grow within the mesophilic temperature range in MRS broth for 4 days at 28, 33 and 37°C. 10 mL of MRS broth tubes were inoculated with 1% of suspected *Lactobacilli* cultures. Periodic (24 hour) measurements of growth was achieved using a UV-VIS spectrophotometer, measurements were taken at 550nm to determine their viability [21].

3. Results and Discussion

Analysis from the isolation of microorganisms from fresh dawadawa using MRS media revealed that of the six visual distinct colonies obtained, they were 2 white, 2 cream, 1 creamy-white and 1 dark cream coloured colonies. The six bacterial strains were given the designations DN, DO, DJ, DT, DG and DH respectively. Morphological examination of these isolates revealed non-spore forming gram positive rod and cocci shaped colonies ranging from creamy to white, clear and transparent with flat elevation (Table 1). Each pure isolate was observed under (40X) microscope. Several reports revealed that a lot of *Lactobacillus* bacterial species which have successfully been isolated from dairy products across Africa appeared as both rod and cocci shaped, non-spore forming and gram positive [14], [22-23]. However, their conclusion was asserted via the results of physiological and biochemical analysis, primarily of which is the catalase test. Just like the studies reported above, this study revealed that all studied isolates were catalase negative, a feature that is synonymous with all known species of *Lactobacillus* [24].

Another principle feature of LABs would be their ability to produce lactic acid as a metabolic by-product from carbohydrate fermentation which is easily assayed via the consumption of lactate [25]. In this study, the isolated bacteria were all capable of consuming lactose, sucrose, trehalose, glucose, maltose and lactulose as the sole carbon source in the broth (table 2). With the exception of isolates

DJ and DG respectively, all other isolates displayed the ability to metabolize dextrose and mannitol. These presents a unique means of identification by screening whereby select strains like *Lactobacillus bulgaricus* from the Lactobacillus family and several from the *Leuconostoc* family display the same biochemical with the inability to ferment mannitol, physiological and morphological features [25]. Growth

optimum studies via pH variation was undertaken and revealed that maximum growth was attainable between 2-3 days of either acidic or alkaline medium (table 3). Analysis of the data obtained indicates growth fluctuations by way of turbidity measurements over time. This addresses the veracity of the cell membrane coupled with the cells acidification activity.

Table 1. Morphological Identification of Obtained Isolates.

S/N	Isolate	Colour	Shape	Opacity	Transparency	Elevation	Margin	Surface	Gram Reaction
1	DN	White	Irregular	Clear	Transparent	Flat	Entire	Smooth	+ve
2	DO	Cream	Circular	Clear	Transparent	Convex	Entire	Smooth	+ve
3	DJ	White	Irregular	Clear	Transparent	Flat	Entire	Smooth	+ve
4	DT	Dark Cream	Irregular	Clear	Transparent	Flat	Entire	Dry	+ve
5	DG	Creamy	Circular	Clear	Transparent	Flat	Entire	Smooth	+ve
6	DH	Creamy-white	Irregular	Clear	Transparent	Flat	Filamentous	Smooth	+ve

Key: +ve = Positive.

Table 2. Biochemical Assessment of Obtained Isolates.

Isolate	Catalase	CO ₂ Production	Sucrose	Lactose	Trehalose	Dextrose	Glucose	Maltose	Mannitol	Lactulose
DN	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
DO	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
DJ	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
DT	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
DG	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
DH	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Key: +ve = Positive, -ve = Negative.

Table 3. pH Analysis and Growth Profile.

Organism	pH 4.5				pH 9.6			
	24Hrs	48Hrs	72Hrs	96Hrs	24Hrs	48Hrs	72Hrs	96Hrs
DN	+	++	+++	+++	+	++	+++	+++
DO	+	++	+++	++	+	++	+++	++
DJ	+	++	+++	+++	+	++	++	++
DT	+	++	+++	+++	+	+++	+++	+++
DG	+	++	+++	++	+	+++	++	++
DH	-	++	+++	+++	+	++	+++	+++

Key: + = low growth, - = no growth, ++ = moderate growth, +++ = Intensive growth, - = no growth.

The isolated bacteria were subjected to the medium containing two concentrations of salt and monitored daily over a 96-hour incubation period. The level of salinity in an environment is a major factor that triggers an osmotic effect of ions on growth. Generally, highly saline environments perturb normal bacteria cell metabolism whereby the increase in salt concentrations lead to cell death via loss of water. However, via natural evolution and diversity studies, it has been observed that some bacteria species initiate growth only in the presence of high salt concentrations. All six strains in this study could grow in the presence of 4.5 and 6.5% NaCl in liquid culture (table 4). The robust physiology of industrially significant lactic acid bacteria towards inhibitory compounds and environmental stress form part of the criteria for selection. In this investigation, growth was monitored in broth cultures containing different concentrations of salt and pH, maintained at 33°C over a 96 hour incubatory period (tables 3 and 4). The data obtained suggested that the generation of by-products such as lactic acid could account

for the conformational changes towards tolerance to aid in its survival in the presence of such inhibitory agents. To survive under such conditions it is assumed that such lactic acid bacteria would have developed mechanism to maintain adequate intracellular pH which may include the reorganization of its lipid composition to thwart the effect of such by-products [26-27].

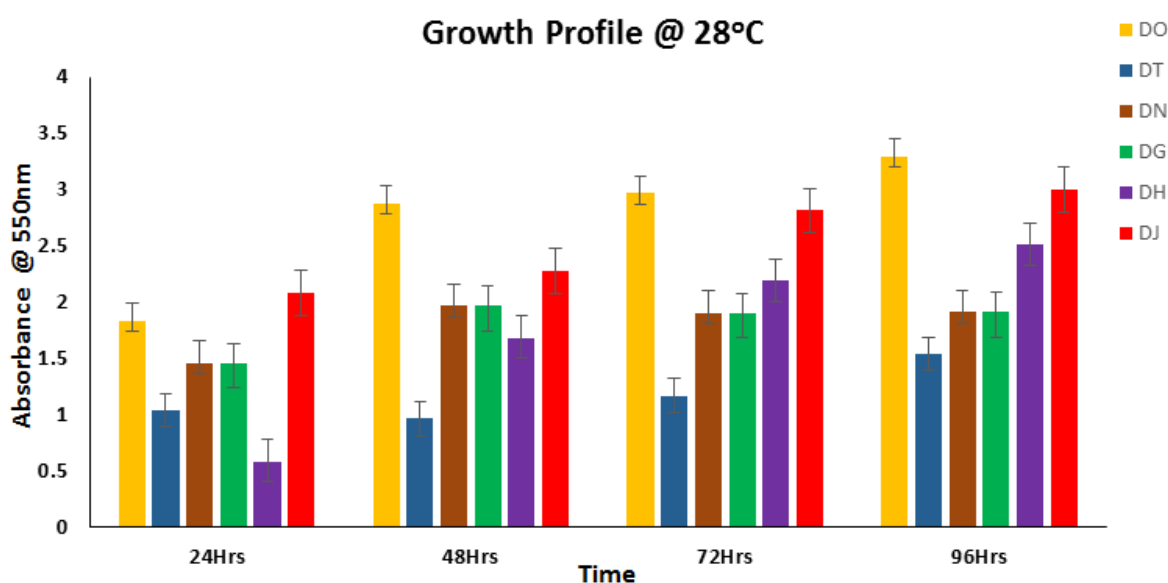
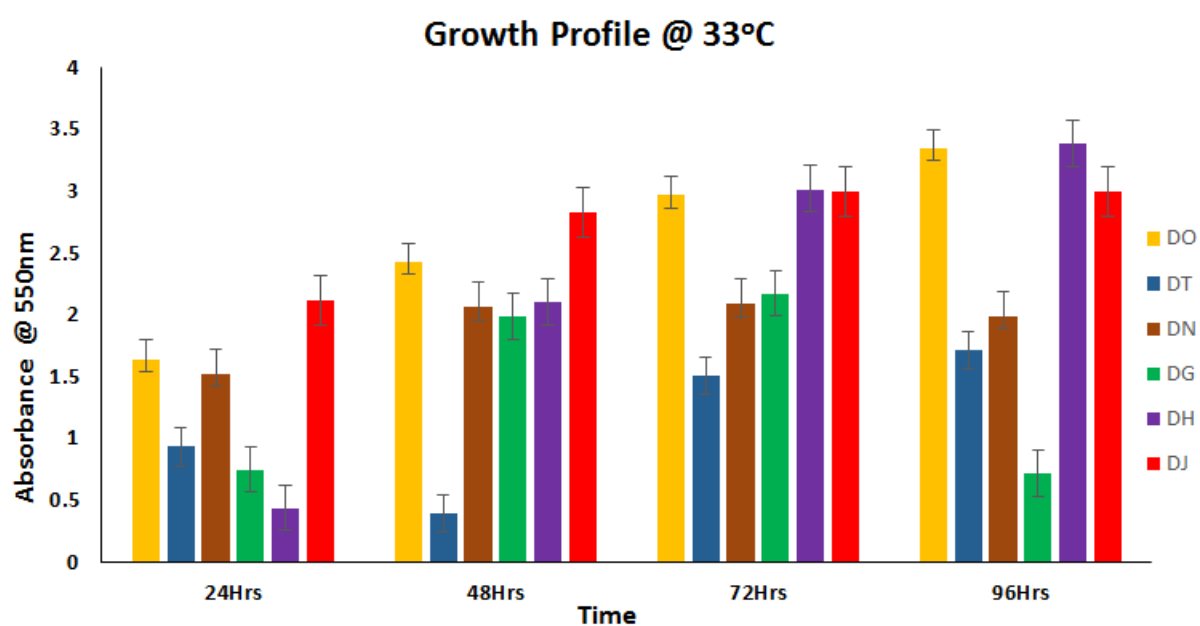
Bacterial growth profile analysis measured at 550nm over a 4 day incubatory period at 28°C, 33°C and 37°C revealed different growth patterns for which all isolates exhibited growth captured by their resulting absorbance values (figures 1-3). Analysis of the growth patterns revealed an almost similar absorbance reading over the three temperatures for which isolate DO and DJ displayed the most prolific rates of growth. Although certain variations were observed between 28-37°C, the values obtained appeared similar to data obtained from other studies [25], [28-29]. The data also suggests that the isolated microorganisms are all mesophilic as their optimum growth is been 20 and 40°C.

Table 4. Effect of Salinity on Bacterial Growth.

Organism	4.5% Conc. NaCl				6.5% Conc. NaCl			
	24Hrs	48Hrs	72Hrs	96Hrs	24Hrs	48Hrs	72Hrs	96Hrs
DN	+	++	+++	+++	+	++	+++	+++
DO	+	++	++	+++	+	++	+++	+++
DJ	+	++	+++	+++	+	++	+++	+++
DT	+	++	+++	+++	-	+	++	+++
DG	+	++	++	+++	+	++	+++	+++
DH	+	++	+++	+++	-	+	++	+++

Key: + = low growth, - = no growth, ++ = moderate growth, +++ = Intensive growth, - = no growth.

These results appear similar to other studies that have successfully isolated and identified different types of lactic acid bacteria from indigenous dairy products across Africa including *Lactobacillus raffinolactus*, *Pediococcus pentosaceus*, *Lactobacillus Plantarum*, *Leuconostoc Mesenteroides*, *Lactobacillus sp.*, and *Lactobacillus acidophilus* [31-32].

**Figure 1.** Growth Determination of Bacteria Isolates over Time at 28°C. Data was obtained from the Mean of Triplicates.**Figure 2.** Growth Determination of Bacteria Isolates over Time at 33°C. Data was obtained from the Mean of Triplicates.

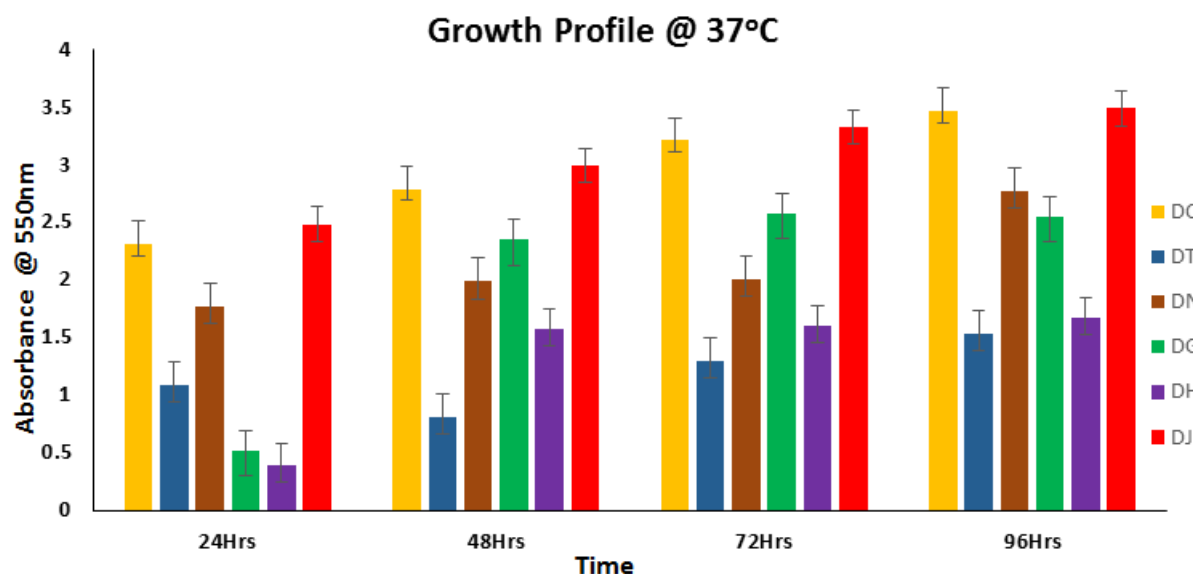


Figure 3. Growth Determination of Bacteria Isolates over Time at 37°C. Data was obtained from the Mean of Triplicates.

4. Conclusion

The overall characteristics of the isolates obtained from dawadawa using MRS media revealed features that are similar to organisms obtained from fermented cow, goat and lamb milk whereby the bacteria all produced acid, were gram positive, catalase negative (Table 1) and capable of survival under microaerophilic conditions [14], [24], [33]. Isolate designated as DO, DT, DN, DG, DH and DJ were all lactic acid fermenters (Table 2) as the predominant characteristic of bacteria obtained from the studied sample. The findings suggest that Lactic acid bacteria are the primary organisms responsible for the fermentation of *Parkia biglobosa* as these organisms were the only dominant bacteria found on the fermenting seeds. As a consumed product, it is suggestive that the microorganisms obtained maybe manipulated towards probiotic as starter cultures in several food and beverage applications. Other results obtained in this study showed that all isolates obtained could thrive at either side of the pH spectrum (Table 3) as a result of the weakly organic acids they produce or the alkaline biomolecules secreted. The close similarities obtained thus far in present study has revealed the availability of indigenous lactic acid bacteria species that possess tolerance to mildly acidic and mildly alkaline environments, thus confirming data obtained in other studies [34].

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