Original Research Article

Isolation of Microorganisms from Fermenting *Parkia biglobosa* in Kuje Market, Abuja

ABSTRACT

Lactic acid bacteria constitute one of the most abundant groups of microorganisms in most fermented food products across Nigeria. The biochemical signatures of these species make the ideal catalysts for a range of processing events with the food industry. This study was undertaken to isolate and screen for lactic acid bacteria strains obtainable from fermented *Parkia biglobosa* seeds purchased at a rural seed market in Abuja. Data obtained revealed that seven morphologically distinct bacteria isolates were obtained using antifungal induced (0.3 mg/ml Fluconazole) yeast extract, malt extract, peptone, glucose, agar media. The seven bacterial isolates were given the designations TEA, TEB, TEC, TED, TEF, TEJ, and TEL respectively. The obtained all but two isolates (TEB and TEL) were all capable of fermenting fructose, glucose, lactose, sucrose and trehalose 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 measurements were determined by measuring the optical density of the cells in broth using spectrophotometer at 600nm over a 96 hour incubatory period in nutrient broth at 37^{o}C. Isolate TEC displayed the highest overall growth pattern over the 24 hour period via optical density readout 1.85, 2.40, 2.65, 2.80. All isolates except TEB were negative for catalase and citrate, thereby suggesting that TEB was a specie of the Citrobacter family. All isolates tested negative for coagulase, indole and motility tests. Suggestions from the data obtained lean towards the use and suitability of the isolated bacteria as ideal cultures for commercial fermentation.

Keywords: Lactic Acid Bacteria, Growth Profile, *Parkia biglobosa*.

1. INTRODUCTION

The ubiquitous nature of microorganisms, particularly its diversity that populates indigenous food groups or materials, is a topic that perplexes the scientific community (Benizri *et al.,* 2002). Although previously conceived that microbial involvement in plants and harvested crops were aligned with their roles in soil fertility (de Boer *et al.,* 2005; Schneider *et al.,* 2012; Massenssini *et al.,* 2015), attention has been drawn to their contribution in improving features such as flavour/taste, texture (exopolysaccharides) and the bio-fortification (cobalamin, folate, riboflavin) of food products (Oyetola *et al.,* 2017). Microbial colonization of food products and/or plant seeds stems from their high nutritional content, thereby creating a plethora of organisms by which researchers have sought to isolate targeted enzymes and other bioactive molecules, suitable for different industrial processes within the food industry (Yelnetty *et al.,* 2014).

African locust bean (*Parkia biglobosa*) is consumed widely within the northern and southwestern regions of Nigeria (figure 1) primarily due to its rich protein content which also serves as an ideal growth medium for the proliferation of diverse microorganisms (Elemo

et al., 2011). It is known by several vernacular names such as nareje in Fulfulde, dawadawa in Hausa*, iru* in Yourba, *ruh* in Yendang, *wupga* in Igala, *néré* in Francophone in Africa (El-Mahmood and Ameh, 2007) illustrating its socio-economic potential in various local communities.

Its identified uses across Nigeria and West Africa include medicinal (47%), food (25%), commercial (22%), handicraft and domestic (3%), veterinary (1%) and cultural (1%) (Koura *et al*., 2011). The bacteria isolated from fermented *Parkia biglobosa* foods have been documented as to enhancing immunity, producing immune-stimulant and displaying probiotic properties such as, hypolipidemic, hepatoproctective and antibacterial; and had been found to be effective in treating gastroenteritis in man and animals (Aderiye *et al.,* 2007). Like most other beans, its rich protein content is nearly indigestible until the bean is fermented (Katz, 2003).

In its fermented state, it is conceivable that lipolytic and proteolytic processes create a uniquely selective environment for a broad diversity of bacterial growth which in the face of food-borne bacterial infections creates the research interest in the biodiversity and ecology of bacteria associated with different foods (Viljoen, 2001).

Figure 1 *Parkia biglobosa* (Jacq.) R.Br. (Fabaceae)

As different species of bacteria are capable of co-existence within and across habitats, which ultimately leads to functional alterations to their physicochemical roles in food, this necessitates the identification of bacterial species associated with different foods. This study aimed at isolating and characterizing bacteria colonizing fermented *Parkia biglobosa* obtained from the Kuje market region of Abuja, Nigeria.

2. Materials and Methods

2.1. Processing of African locust bean (Parkia biglobosa) seeds

Parkia biglobosa seeds were obtained from the National Seed Council, Sheda, Abuja with the voucher number given. Additional seeds were also collected from Kuje market of Abuja, Nigeria. Each seed sample from their respective collection point was placed in sterile labelled containers separately and placed in a polyethylene bag during transportation to the laboratory employing standard conditions for sample collection.

A measured amount (400 g) of the beans was washed with distilled water and boiled for 10 minutes at 121^{o}C at 100 kpa atmospheric pressure, the cotyledon was removed and rewashed with distilled water. To the bean sample collected from the Seed Council, 50g was placed aseptically in a sterile bottle inside a bio-safety cabinet wish serve as control and 300g of each market collected bean was placed in a calabash covered with fresh banana leaves to induce local fermentation in a clean cupboard. Samples were picked daily for microbial isolation.

2.2. Isolation of microorganisms

Five grams (5 g) of ground bean samples were suspended in 10 ml distilled water in sterile bottles with vigorous shaking. The suspension was ten-fold diluted and inoculated using pour plate method on sterile Muller Hinton agar, Nutrient agar and on YPMG (Yeast extract,

peptone, malt extract, glucose, agar agar) supplemented with 0.3 mg/ml fluconazole for 18 to 24 hours and observed for the growth of colonies. All experiments were carried out in triplicate.

2.3. Identification by Morphological and Biochemical Assessment

Visually distinct colonies were randomly selected and streaked repeatedly on anti-fungal supplemented YPMG 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 (Barnett *et al.,* 2000). Wet mounts of isolated cell samples were prepared in distilled water and examined using 40X objective magnification.

Biochemical characterization performed included Gram reactions, catalase, citrate, indole, oxidase, Voges-Proskauer and Methyl-Red test, fermentation of carbohydrate (galactose, glucose, lactose, sucrose, mannitol, fructose and starch) following known procedures (Ayeni et al., 2011).

2.4. Determination of Growth Optima

Fresh 24 cultures of each respective isolates were inoculated onto streaked on sterile nutrient broth and incubated at 37°C for 96 hours. Samples were monitored daily for growth and analyzed using a UV-VIS spectrophotometer in order to ascertain their viability.

2.5. Thermo-tolerance studies

Thermo-tolerance assessment was determined by inoculating individual bijou bottles containing 10ml autoclaved nutrient broth with 1% of each bacterial culture then incubated at different temperature controlled environments (25^{o}C, 30^{o}C, 37^{o}C, 45^{o}C). The optical density of each bijou bottle was recorded on a spectrophotometer at 600 nm against the medium (nutrient broth) as blank.

3. Results and Discussion

Seven bacterial strains were successfully isolated from the fermented. Analysis from the isolation of microorganisms from fresh dawadawa (commonly referred to as iru) using MRS media revealed visually distinct coloured colonies; white, cream, creamish-white and dark cream, with the designations TEA, TEB, TEC, TED, TEF, TEJ and TEL respectively (Table 1). 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). The further morphological assessment revealed that each isolate appeared as flat and smooth textured with a transparent opacity (Table 1). Microscopic examination was achieved during the growth phase. All the strains were gram positive (Table 1). Each pure isolate was observed under (40X) microscope. Microbial count analysis revealed that the maximum number of colonies obtained via plate count method was 4 x 10^6 colony forming units per ml while the least number was 5 X 10^4 colony forming units per ml (Table 1). Within the last two decades, several reports have described the colonization of indigenous fermented food stuffs by a vast array of *Lactobacillus*, whose morphological appearance upon isolation were predominantly rod and cocci shaped, non-spore forming and gram positive (Savadogo *et al.,* 2004; Moulay *et al.,* 2013).

Biochemical assessment of all seven organisms revealed all isolates were catalase negative with the exception of isolated with designation TEB (Table 2). Data also revealed that all isolates bar

TEB and TEL possessed the inherent ability to utilize lactose, whereas all isolates demonstrated good ability to consume glucose (Table 2).

Both biochemical and physiological assessment of isolated bacterial species is extremely important as the outcome of peculiar tests such as catalase proffers a strong indication towards specie diagnostics. To this end, it has been reported via process (biochemical) elimination that a common feature of all know species of *Lactobacillus* (Khalid, 2011).

Growth profile of the microorganisms over a four day incubation period was monitored in the broth samples at room (37^{o}C) temperature (Table 3). Analysis of the growth pattern exhibited continuous growth for all but isolate designated TEC displayed an almost similar growth maximum over the incubatory period with for all isolates. Isolate TEC displayed the highest overall growth pattern over the 24 hour period (Table 3). The results obtained appeared similar to values obtained from other related studies, thus suggestive that isolates designated TEA to TEL are predominantly mesophilic microorganisms (Begum *et al.,* 2017).

Table 1. Morphological characteristics of Bacteria isolated from *Pakia biglobasa* beans collected at Kuje market

S/N	Code	Plate Count	Color	Elevation	Gram reaction	Margin	Surface	Microscopy observation
1	TEA	$6 X10^{5}$	White	Raised	Positive	Smooth	Filiform	Rod Single and in chain
2	TEB	$5 X 10^{4}$	Cream	Raised	Positive	Smooth	Spreading	Rod with rounded end
3	TEC	$7 X 10^{4}$	Cream	Raised	Positive.	Wrinkled	Beaded	Cocci single pairs and chain
4	TED	$4 X 10^{5}$	White	Raised	positive	Rough	Small beads	Rod and in chain
5	TEF	$8 X 10^{4}$	White	Convex	positive	Smooth	Spreading	Cocci, single and chain
6	TEJ	$6 X 10^{4}$	Light cream	Raised	Positive	Wrinkled	Breaded	Cocci, single and pair chain
7	TEL	$4 X 10^{6}$	Cream	Flat	Positive	Smooth	Spreading	Rod with rounded end

Following the principle biochemical feature of *Lactobacillus* species which is the ability to metabolize lactose as the sole carbon source, it is easily deduced that isolates TEB and TEL were the only non-Lactic acid bacteria whereby the biochemical characteristics of isolate TEB were identical to that of *Citrobacter diversus*, according to the Bergeys manual of bacteriology (Begum *et al.,* 2017).

Based on the ability to, metabolize glucose, sucrose, mannose, fructose, arabinose, and trehalose as the respective sole sources of carbon in the broth cultures, the predominant species identified were *Lactobacillus bulgaricus, Lactococcus lactis, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus helveticus and Lactobacillus delbruecki* (Table 2).

Table 2. Biochemical characteristics of Bacteria isolated from locally fermented *Parkia biglobosa* bean

Code	Catalase	Coagulase	Citrate	Glucose	Lactose	Sucrose	Mannose	Fructose	D-Arabinose	Indole	Motility	Trehalose
TEA	**Nil**	**Nil**	**Nil**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**Nil**	**Nil**	**+ve**

TEB	+ve	Nil	+ve	+ve	Nil	Nil	+ve	Nil	+ve	Nil	Nil	Nil
TEC	Nil	Nil	Nil	+ve	+ve	Nil	+ve	+ve	+ve	Nil	Nil	+ve
TED	Nil	Nil	Nil	+ve	+ve	+ve	Nil	AG	Nil	Nil	Nil	+ve
TEF	Nil	Nil	Nil	+ve	+ve	+ve	Nil	+ve	Nil	Nil	Nil	+ve
TEJ	Nil	Nil	Nil	+ve	+ve	Nil	Nil	Nil	Nil	Nil	Nil	+ve
TEL	Nil	Nil	Nil	+ve	Nil	+ve	+ve	+ve	+ve	Nil	Nil	Nil

Table 3. ***Growth Profile from*** **bacteria isolated from locally fermented** ***Parkia biglobosa*** **bean**

Code	24 Hrs	48 Hrs	72 Hrs	96 Hrs
TEA	1.95 ± 0.15	2.20 ± 0.50	2.35 ± 0.30	2.55 ± 0.10
TEB	1.80 ± 0.35	2.35 ± 0.15	2.45 ± 0.45	2.65 ± 0.45
TEC	1.85 ± 0.40	2.40 ± 0.35	2.65 ± 0.15	2.80 ± 0.35
TED	1.65 ± 0.10	2.30 ± 0.25	2.65 ± 0.65	2.85 ± 0.30
TEF	1.90 ± 0.25	2.15 ± 0.35	2.30 ± 0.50	2.55 ± 0.25
TEJ	1.70 ± 0.80	2.05 ± 0.20	2.25 ± 0.25	2.50 ± 0.15
TEL	1.65 ± 0.60	2.10 ± 0.65	2.40 ± 0.35	2.60 ± 0.20

Table 4. Determination of thermo-tolerance ***for*** **bacteria isolated from locally fermented** ***Parkia biglobosa*** **bean**

Code	25°C	30°C	37°C	45°C
TEA	1.80 ± 0.75	2.30 ± 0.30	2.45 ± 0.10	--
TEB	1.65 ± 0.90	2.10 ± 0.20	2.20 ± 0.15	--
TEC	1.90 ± 0.25	2.20 ± 0.55	2.35 ± 0.65	--
TED	1.85 ± 0.25	2.45 ± 0.15	2.70 ± 0.45	--
TEF	1.90 ± 0.60	2.25 ± 0.10	2.45 ± 0.20	--
TEJ	1.65 ± 0.55	2.00 ± 0.65	2.10 ± 0.45	--
TEL	1.70 ± 0.40	2.20 ± 0.40	2.55 ± 0.15	--

4. Conclusion

From the data obtained in this study, the microorganism obtained from *Parkia biglobosa* seeds revealed characteristics that were synonymous with organisms obtained from several other milk products such that the bacteria were gram positive, catalase negative (Tables 1-2) and thrived well at room temperature (Savadogo et al., 2004; Khalid,

2011; Yelnetty *et al.,* 2014). The results obtained suggest that Lactic acid bacteria are the predominant species that colonize fermented *Parkia biglobosa* seeds.

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