Research Paper
Amajor and Amajor, V-8; I (2): 88-104 (2020)

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Journal of biotechnological sciences (ISSN: 2308-0043)

Molecular Identification of Lactic Acid Bacteria Strains Isolated during natural Fermentation of newly bred yellow cassava dough.

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Abstract
The study aimed to isolate and characterize from the fermenting newly bred yellow cassava dough by PCR-based molecular methods to identify the isolates to species level which cloud facilitate to formulate starter cultures and help in the preservation of foods. The yellow cassava dough was fermented and 30 isolates were isolated during fermentation at different stages at National Root Crops Research Instutite, Umudike. Methodology: The lactic acid bacteria isolates were selected on the basis of positive to acid production,(Gram stain reaction- positive, Catalase-negative, Oxidase- negative, Cell form- rod shaped). PCR- based molecular methods were used to identify the LAB isolates. A total of 30 lactic acid bacteria (LAB) isolates were obtained from fermenting dough of the newly bred yellow cassava and characterized at species level. The DNA of the bacterial isolates were extracted using ZYMO KIT (zymo research group California, U.S.A). 16s rRNA of the isolates were amplified using primiers 27F and 1492R and the amplified product sequenced at Ingaba Biotech West Africa (IBWA) South Africa. Sequence analysis were compared within the resource at database and were carried out to identify the isolated microorganism. The result showed 30 isolates and 10 each group of LAB. They were identified as LAB with rod shaped cell forms, Gram stain- positive, oxidase-negative, glucose- positive and negative and positive acid production. The 16s rRNA gene sequencing showed all the isolates. The bacterial LAB isolates were identified as Lactobacillus brevis , Lactobacillus plantarum and Lactobacillus fermentum with similarity index of 100%. The indigenous LAB of newly bred yellow cassava dough cloud be a potential source of starter cultures and biological preservation of food.

Key Words: Fermented dough; yellow cassava; Lactic acid bacteria; PCR; 16s rRNA gene; phylogenetic tree.

Introduction

Fermentation is the conversion of carbohydrate into alcohols and short chain fatty acids by enzymes of microorganisms as stated by (Silva *et al.,* 2008).The primary benefit is the conversion of sugars and other carbohydrates to usable products.

In processing of foods, fermentation has been identified as an economic processing method that could be used in the homes to improve the nutritional quality of plant foods (Obizoba and Atti, 1991; Apata and Ologhobo, 1990). About 90% of the cassava processed in Africa is used for human nutrition as fermented food products according to Mensah (1997). Hence, fermentation is the most popular method used for cassava processing. Fermentation have shown to a better method to enhance the safety, organoleptic and nutritional quality of many cassava food products as reported by (Oyewole, 1997).

In some parts of the world, huge amounts of fermented foods are produced and consumed daily diet of the people (Steinkraus, 1995). Fermentation has been used quite extensively in various parts of the world especially in the orient (Bressani, 1983). It involves the activities of microorganisms (bacteria and fungi) in the production of food. Fermentation could be done at home or in industry. In Nigeria, fermentation of foods play a major role in the diet of the people (Steinkraus, 1994). Some of these include gari from cassava, ogi from corn, flour from fermented cassava, pito beer from millet/sorghum and different kinds of condiments. The fermented foods are derived from substrates like roots, legumes, cereals, oil seeds, nuts, meat, fish, milk, palm tree sap etc.

Fermented foods are usually prepared from plant or animal materials by processes in which micro-organisms play important role in modifying the substrates physically, nutritionally and sensorily (Aideo, 1986). Many fermented products have the advantage of prolonged shelf life due to the organic acid produced during fermentation which is fatal to diverse spoilage micro-organisms (Odunfa, 1985). The acceptability of such fermented products is important in practical nutrition. It is known that the nutritional advantages of a food, the relative cost, availability and organoleptic acceptability are the major factors (Eka, 1984).

Fermentation of cassava dough by bacteria and yeasts does not only improve detoxification; it also improves food quality and safety by product preservation, flavor development, cyanide reduction and changes in functional properties as reported by Obilie *et al*., (2003).

Cassava (Manihot esculenta Crantz) is the third agricultural resource after rice and maize as a source of calories in tropical countries (FAO, 2008; De Oliveira *et al.,* 2015; Ngobisa *et al.,* 2015). In Africa, cassava is one of the most important food crop (FAO, 1999) and Nigeria is the current leading cassava producing country in the world (FAO, 2002). In Nigeria, cassava is consumed in boiled, baked and fried forms in addition to various other products that are gotten from fermenting the crop. Cassava root is normally processed before consumption so as to detoxify, preserve and modify them (Oyewole, 1991). Among the fermented cassava products of cassava roots are "gari", "fufu", "lafun", "pupuru" and "tapioca" (Lancaster *et al*, 1982) In Nigeria, cassava is processed into gari, fufu, pellets for compounding animal feeds, kpokpo gari and also into instant aromatized (fermented) flour (Oyewole, 1991). As at date, new cassava varieties are being introduced to farmers for their agronomic benefits with little considerations for the quality of the end products (Akoroda, 1992). Cassava roots contain mainly carbohydrate of which 80%

is starch, the level of protein (1-2%) and fat less than 1% are not nutritionally significant (Goomez *et al.*, 1984). Cassava is traditionally processed into a wide variety of fermented products such as attiéké, gari, lafun, fufu, baton de manioc or chips, particularly suited to transportation, trade and rapid preparation of meals (Kouamé *et al.*, 2012). Over the years the demand for the production and consumption of fermented foods has extremely increased and accordingly, those foods occupied a substantial part of the diet worldwide (Elyas *et al.*, 2015; Ngobisa *et al.*, 2015).The new bred varieties acts as vehicle of conveying pro-vitamin A to vitamin deficient (VDA) population as reported by Tanumihardjo *et al*. (2008). The fresh bio-fortified cassava roots are also referred to as yellow cassava. The yellow cassava according to Egesi (2011) is a newly released bio-fortified crop which is similar to the white varieties in terms of utilization for man and animal, though the pulp colour differs. Vitamin A remains a very important component of human nutrition as it is involved in vision, cell differentiation, synthesis of glycoproteins, reproduction and overall growth and development (Woolfe, 1992).

Lactic acid bacteria (LAB) are made up of a heterogeneous group of Gram-positive bacteria and are widely consumed along with fermented foods and beverages because of their use as starter cultures in fermentation processes according to Abdelgadir, *et al*. (2008). They colonize the gastrointestinal and urogenital tracts of humans and animals and are present in foods such as dairy products, fermented meats, fermented dough, fruits and vegetables (Adedohe and Babalola, 1988). Some LAB species are classified as "Generally Recognized As Safe" (GRAS) by the United States Food and Drug Administration (FDA) Elyas *et al.*, (2015) or have the "Qualified Presumption of Safety" (QPS) status by the European Food Safety Authority. Accordingly, certain species of these genera are intentionally added to several probiotic products due to their potential health benefits (AI-Jahiz,1980). The genus Lactobacillus is the largest group among the Lactobacteriaceae and contains over 100 species (Albesharate, *et al.* 2011).They are characterized as Gram-positive rods, anaerobic but aero tolerant, non-sporulating and catalase negative. They are commercially used as starter cultures in the manufacture of dairy products, fermented vegetables, fermented dough, alcoholic beverages and meat products (Amoa-Awua *et al*.1996).

Lactic Acid Bacteria (LAB) involved in cassava dough production not only are responsible mainly for acidification but also play relevant roles in the modification of flavour and texture of the products and inhibition of spoilage and pathogenic microorganisms (Ashraf *et al*.2009). In this context, the studies have been focused to the characterization and identification of LAB from the newly bred cassava dough production (De vos *et al*.2009; FAO/WHO 2001), which is considered essential for understanding the contributions of LAB to cassava dough production. Today, with growing urbanization, cassava have become a commercial home-based industry in Nigeria. Recently, it has been shown that *Lactobacillus* and *Saccharomyces* cultures can be used to reduce the fermentation time from 96-48 h, (Oduah *et al*.2015) which would be useful for commercial production. In general, the classical protocols of morphological and biochemical characterizations of microbial cultures are in use to identify isolated bacterial culture. The development of PCR-based methods using random amplification of polymorphic DNA (RAPD) (Dirar, 1993) analysis of 16S rRNA gene homology, amplified (Dirar, 1994) and species specific primers (EFSA, 2012), have proved useful for identification of various species of LAB. Thus, the present study was focused on isolation and characterization of LAB from fermented cassava dough by PCR-based molecular methods to identify the isolates to sub-species level which may help to formulate starter culture as well as in the biological preservation of foods.

Preparation of fermented yellow cassava roots

Healthy roots of 12 months (TMS O1/1368) umucass36 cultivars of yellow cassava were harvested from the production field of National Root Corps Research Institute (NRCRI) Umudike and taken to the laboratory for processing. About 40kg of the cultivar were used for production of Garri. The roots were processed using the methods described by Aniedu and Oti (2007). The roots were peeled manually and washed with clean water. The washed roots were cut into 3.5-4.0 mm thickness and was subjected to traditional fermentation for 72 h in plastic container of same volume and diameter (25 liters and 54cm) respectively and labeled. It was soaked and allowed to ferment for 72h room temperature.

Isolation of lactic acid bacteria (LAB)

At 24 h intervals, 5 ml of the fermenting yellow cassava mash was collected from each of the samples and dispersed into 45 ml of sterile distilled water in the test tube as aliquots. The mixtures were shaken to homogenize. Serial dilutions were carried out using 10-fold dilutions. The pour plate method as described by Ezeama, (2007) was used. Aliquot (1.0 ml) of the samples were introduced into each sterile petri dish, then 15 ml of liquefied MRS agar at 45^0C was poured into each of the plates and mixed by rotating the plate first in one direction and then in the opposite direction. The agar on the plates were allowed to solidity on a level surface and plates incubated aerobically at 37^0C using the anaerobic Jar (gas pak system, BBL) at 30^0C for 72h. The colonies were observed and counted and expressed as colony forming unit per gram (Cfu g-1). Specific counts of lactic acid bacteria were cultured on plates of MRS agar (oxoid). The plates were incubated at 30^0C for three days before counting. Pure cultures were stored for further use on slants at 40^0C.

Each of the lactic acid bacteria isolated from natural fermentation were examined for phenotypic characterization which were positive to gram staining, catalase test, oxidase test and acid test. The lactic acid bacteria isolates were further confirmed using molecular characterization at International Institute of Tropical Agriculture (IITA) Ibandan, Nigeria.

Characterization of the LAB isolates: The 30 lactic acid bacterial isolates were characterized according to their morphology by Gram staining and shape under microscope (cell formation) and biochemistry by presence or absence of catalase, oxidase, and gas production (glucose) as described by Vos *et al*. (2009). Molecular characterization was carried out at International Institute of Tropical Agriculture (IITA) Microbiological Unit Ibadan, Nigeria and DNA sequencing was done at Ingaba Biotech West Afroca (IBWA) South Africa in Collaboration with IITA. Ibadan Nigeria. Sample processing, laboratory isolation and identification of (LAB) were carried out at National Root Crops Research Institute Umudike, Nigeria. Acid production test were done by the method described by (Dicks and Van-Vuren, 1987) with slight modification. Each strain was inoculated at 30^0C in 5ml MRS agar medium without beef extract and with 0.004 g/l of bromocresol purple in tubes. Acid production was monitored for three days, for yellow area formation in the tube and the acidification ability was analyzed by a visual examination of the yellow area's spread.

DNA extraction of lactic acid bacteria (LAB) isolated during Traditional fermentation of yellow cassava variety.

DNA extraction Whole genomic DNA was extracted from the LAB samples using the ZYMO kit (Zymo research group California USA). Using the following steps:

1. Microorganisms (LAB) were cultured on MRS agar and incubated for 24 h.
2. Hundred milligrams (100mg) of the cultured microorganisms was collected and suspended in 200µl of water in an eppendorff tube and 750 ml of bashing bead lysis solution was added.
3. Bashing bead was added and the Eppendorf tube was secured in a 2 ml tube holder Distruptor Genie (cat no. s6001-2120 from Zymo Research corp.) and processed at maximum speed for 5mins.
4. The bashed sample was centrifuged at 10,000 Xg (r.p.m) for 1 min
5. 400µl of the supernatant was transferred to a Zymo-spin filter in a collection tube and centrifuged for 1min at 8,000X g (r.p.m).
6. Genomic lysis buffer (1200µl) was added to the filtrate in the collection tube
7. An aliquot of 800µl of the above mixture was transferred into the zymo –spin IIC Column in A collection tube for 1min at 10,000X g (r.p. m).
8. The flow thru was discarded and 200 µl of DNA pre-wash buffer was added to the above column after which it was spinned for 1min at 10,000X g (r.p.m).
9. 500µl of g-DNA wash buffer was then added to the column and spinned at 10000Xg for 1 min.
10. The column was then transferred to a new Eppendorf tube and 100µl of DNA elution buffer was added to elute the DNA. The ultra –pure DNA which is suitable for PCR and other purposes) was then transfered into a -20^{0}C fridge for storage. (Zymo research group California USA). Aliquots of the Extracted DNA was mixed with loading dye (Thermo scientific Loading dye) and was loaded on 1% agarose gel (Fig 1 and Fig 2) using SB buffer and run using the following electrophoresis conditions 121v, 120 mA for 1 hour after which it was placed in a gel doc (Multi Doc –it digital imaging system) so as to visualize the bands. DNA Gel pictures below in figure 1: Extracted DNA was then amplified using the broad –range 16s rRNA PCR (Amani et al., 2017) on the V4 region using the primers: For Bacterial samples (LAB strains): 27F: 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492R: 5'-GGTTACCTTGTTACGACTT-3' (Yi-Lin et al., 2015).

PCR CONDITIONS for 16 s rRNA

Initial den.	**Den.**	**Ann. Tempt**	**Extension**	**No. of circles**	**Final extension**	**Hold tempt**
94˚C	94˚C	50˚C	72˚C	40	72˚C	4˚C
3min	45sec	60sec	90sec		10min	∞

PCR Cocktail mix

The DNA was subjected to the following cocktail mix and condition for the PCR

10× PCR buffer	1.0
25mM Mgcl2	1.0
5pMol forward primer	0.5
5pMol reverse primer	0.5
DMSO	1.0
2.5Mm DNTPs	0.8
Taq 5u/ul	0.1
10ng/µl DNA	2.0
H2O	3.1
	10µL

The amplicon from the 16s rRNA PCR reaction above was loaded on 1.5% agarose gel and the gel picture is attached as PCR. The ladder used is hyper ladder (50bp) from BIOLAB. The expected base pair of the amplicon is around 750bp). (Fig 1 and 2).

DNA Sequencing and BLAST Search

DNA sequencing was sent to Inqaba Biotech West Africa (IBWA) (a subsidiary of Inqaba Biotechnical Industries (Pty) Ltd. P. O Box 14356, Hartfield 0028 Pretoria, South Africa) for sequencing which was carried out using the Sanger method with an Applied Biosystems (Foster City, CA, USA) automatic DNA sequencer (ABI PRISM 3130x l Genetic Analyzer) and an Applied Biosystems BigDye (ver. 3.1) kit. Blast search (Altschul *et al.*, 1990) was carried out using the NCBI nucleotide database "16S rRNA sequences (Bacteria and Archaea)" with the program selection optimized for "Highly similar sequences (megablast)."This was done by a manual blast search against the NCBI 16S isolate database for the top 10 operational taxonomic unit species (OTUS) for exploratory purposes (NCBI BLAST, RRID: nlx_84530). Results from the operational taxonomic unit (OTU) clustering were matched to the Greengenes data base using an RDP classifer within the QIIME pipeline and used for further analysis.

Results and Discussion

Phenotypic Identification of LAB: Thirty pure isolates were obtained from yellow cassava fermented mash and were identified as LAB based on their morphology, and biochemical tests. All isolates were Gram-positive, catalase and oxidase negative. All isolates grew on MRS agar under aerobic conditions, and indicating that the LAB isolates from the study were found to members of the genus Lactobacilli. The morphological and biochemical profiles of isolated LAB were shown in Table 1.

Characterization of the isolates: The morphological characteristic of the LAB isolates were identified by Gram staining, which showed that all isolates were Gram-positive bacteria. The analysis of the shapes of the LAB showed that all isolates showed the rod shaped characteristic (Table 1). The test of the biochemical characteristics showed that all isolates were catalase-negative, oxidase-negative and 74% of the LAB could produce gas from glucose, indicating that 83% of the LAB isolates were heterofermentative Lactobacilli and 16% were homofermentative *Lactobacilli.* Lactic acid (LAB) is divided into homofermentative and heterofermentative bacteria based on their ability to ferment glucose as reported by Setiawan *et al* (2017). The heterofermentative LAB could produce gas as an end product of fermentation

The morphological profile of the isolates (LAB)

Isolated codes	Shape	Gram stain reaction	Catalase	Oxidase	Glucose	Acid production
LP1A	Rod	+	-	_	+	+
LP1A	Rod	+	-	_	+	+
LP1B	Rod	+	-	_	+	+
LP1C	Rod	+	_	_	+	+
LP1C	Rod	+	_	_	+	+
LP2A	Rod	+	_	_	+	+
LP2B	Rod	+	_	_	+	+
LP2B	Rod	+	_	_	_	+
LP2C	Rod	+	_	_	+	+
LP3A	Rod	+	_	_	_	+
LB1A	Rod	+	_	_	+	+
LB1A	Rod	+	_	_	+	+
LB1B	Rod	+	_	_	+	+
LB2A	Rod	+	_	_	+	+
LB2A	Rod	+	_	_	+	+
LB2B	Rod	+	_	_	+	+
LB3A	Rod	+	_	_	+	+
LB3B	Rod	+	_	_	+	+
LB3B	Rod	+	_	_	+	+
LB3B	Rod	+	_	_	+	+
LF1A	Rod	+	_	_	+	+
LF1A	Rod	+	_	_	+	+
LF1B	Rod	+	_	_	+	+
LF2A	Rod	+	_	_	_	+
LF2B	Rod	+	_	_	+	+
LF2C	Rod	+	_	_	+	+
LF2C	Rod	+	_	_	+	+
LF3A	Rod	+	_	_	_	+
LF3A	Rod	+	_	_	_	+
LF3B	Rod	+	_	_	+	+

LAB: lactic acid bacteria; +: Positive;- : Negative

16S rRNA gene sequences and phylogenetic analysis: The LAB isolated form yellow cassava dough will be more accurately identified to the species level by 16S rRNA gene sequencing. In this study, the 16S rRNA gene sequences were successfully amplified from 3 groups of isolates by Polymerase Chain Reaction (PCR). The identification of the lactic acid bacteria (LAB) isolated

during traditional fermentation was done based on 16s rRNA gene seqencing. The isolates from the fermenting mash were identified as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus berivs.* All the LAB isolated in this study were found to be members of Lactobacilli Genus. A total of 30 lactic acid bacteria were isolated from (umucass 36) cassava dough and were analyzed in 10 groups and they were mainly genus of *lactobacillus*. The most frequently isolated LAB species from the mash were *L. plantarum* .The 16s rRNA gene sequences of 750bp of all isolates when compared with related bacteria using the BLAST Program at NCBI (http://www.ncbi.nih.gov/) showed that the isolates were closest relatives of *L. plantarum, L. fermentum, L. berivs* which showed 100% proximity. The study indicates the presences of *L . plantarum, L. fermentum,* during the fermentation of cassava starch as stated by Lacerda, *et al*;(2011).

Electrophoresis for DNA bands are shown in fig 1 and 2 using the primers For Bacterial samples (LABstrains): 27 F: 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492 R: 5'-GGTTACCTTGTTACGACTT-3' (Yi-lin *et al*.,2015).

Figure:1: The PCR amplification was performed to obtain more copies of the 16s rRNA gene for the sequencing process as shown below indicating 99-100% sequence similarity to *L. platarum, L. fermentum, L. breivs.*

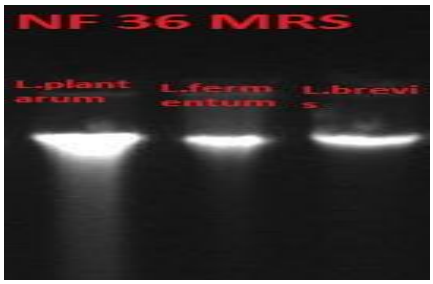
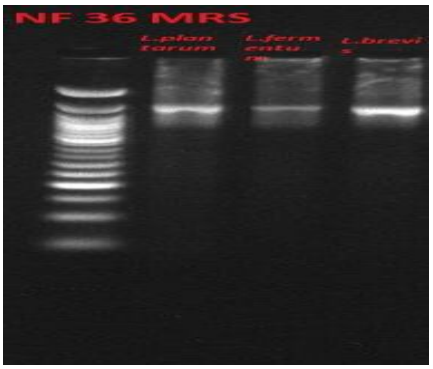


Figure:2



Lactobacilli represent one of the major microbial groups involved in these desirable fermentations. Among them, *Lactobacillus plantarum* is known among cassava dough fermentative germ as reported by Kostinek *et al.,* (2007). The LAB can also be developed into probiotics for functional food. Probiotics are living micro-organisms that if in enough amount in human or animal will confer health benefits to the host by improving the intestinal microbiota balance (Alegria *et al.*, 2016).

The dominant species *L. plantarum* frequently occurs (spontaneously) in high numbers in most fermented foods, especially when the food is based on plant material as reported by Kawthar *et al* (2018). The LAB strains obtained during the study could act as potential pure microbial starter for cassava dough fermentation in to *Garri.* The breaking down of the coarse texture of the cassava roots was critical for the the softening of cassava tissue, which improves the quality of the cassava food products as reported by Amoa-awua and Jakobsen (1995). The softening of cassava tissue is achieved by LAB which produce cellulose and pectinase in cassava dough. The microbial β-glucosidase activities are also vital to detoxify the cassava dough by hydrolysis of linamarin available in the plant cells. The 30 isolates of LAB showed good acid production on the basis of their biochemical properties. The LAB convert glucose to organic acids that caused pH reduction ± 4.4. LAB played an important role during the starch hydrolysis for production of the sour starch product as reported by Obilie (2004). The LAB obtained in study namely *L. brevis, L. plantarum. L. fermentum* were prevalent throughout the entire process. This is similar to the result obtained by Fernanda et al. (2018) in identification of micro- organisms from cassava flour. Among the LAB often found in traditional cassava fermentations, many authors report the presence of *L. plantarum, L. brevis* and *L. fermentum* as reported by Kostinek *et al*.(2007); Oguntoyinbo,(2007). Lacerda *et al.* (2005) reported L. *Plantarum and L. fermentum* as the predominant LAB during sour starch fermentation isolated from two cassava flour manufacturers and *L. brevis* was isolated in minor numbers. The population of indigenous LAB tend to dominate sour dough fermentations by the production of acid in the fermented dough according to Kawthar *et al* (2018). Majority of LAB isolated in this study were homofermenters. This is in agreement with other research workers who reported the predominance of obligately homofermentative LAB in fermented maize meal for the production of sour bread and homofermentative lactobacilli and Pediococcus spp. from the final sour dough for production of Swedish rye bread Kawthar *et al* (2018).

Lactic acid bacteria (LAB) provides health benefits to human, animal and plant according to Song *et al*. (2012). Their nutritional needs may vary among species and even among strains (Hebert, *et al.*, 2004) Lactic acid bacteria strains are fast growing organism which can utilize different metabolic activities which is related with production of compounds that are beneficial such as antimicrobial compounds and organic acids, enzymes which can breakdown complex organic compounds into simpler functional compounds (Vonwright and Axelesson, 2011). The lactic acid bacteria benefits and application is based on its quick growing characteristics and the metabolic activity.

Fig .3 The *L. fermentum* phylogenetic tree based on partial 16s rDNA sequences showing the relationship between isolate and other species belong to the genus *lactobacillus*

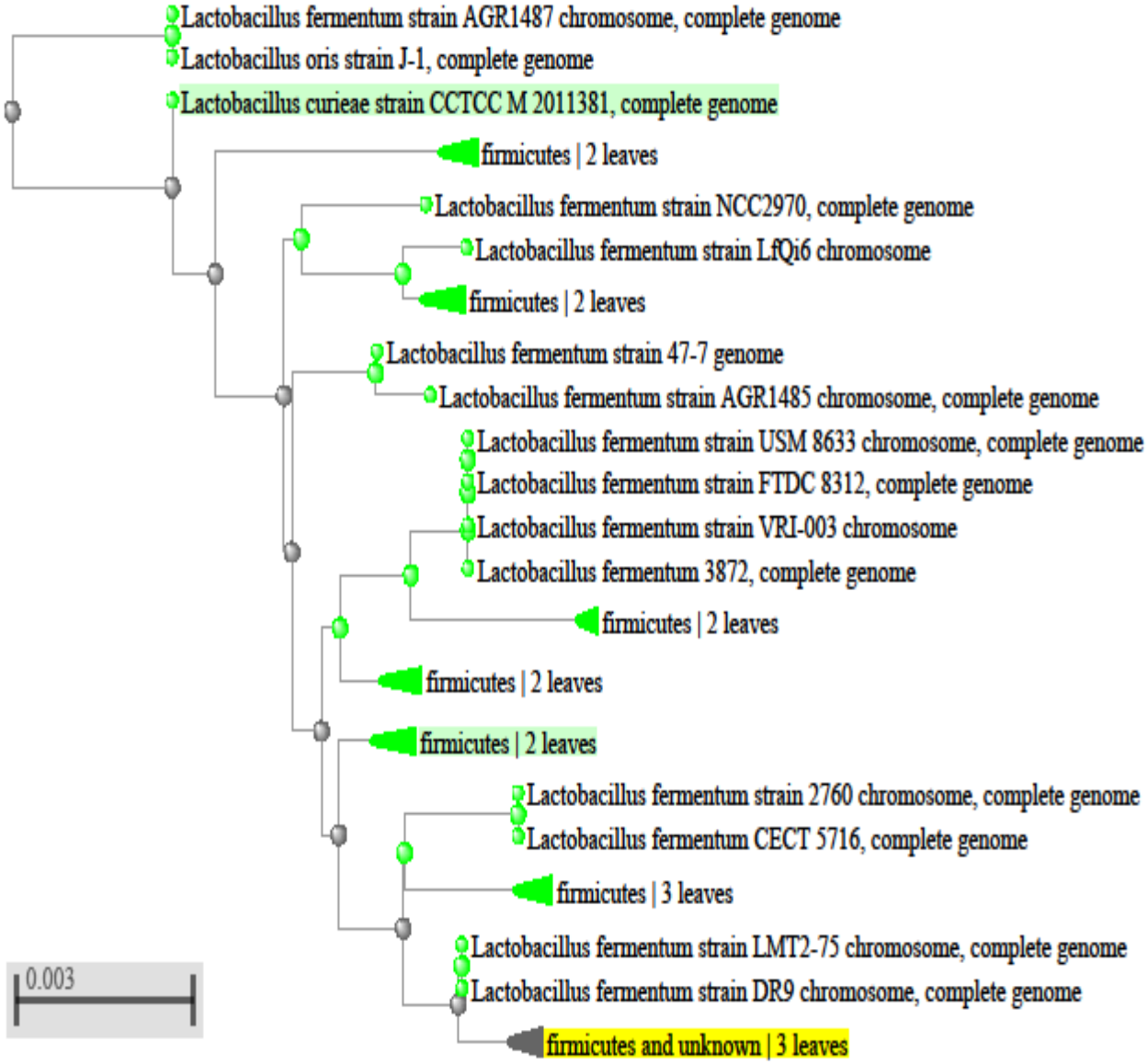


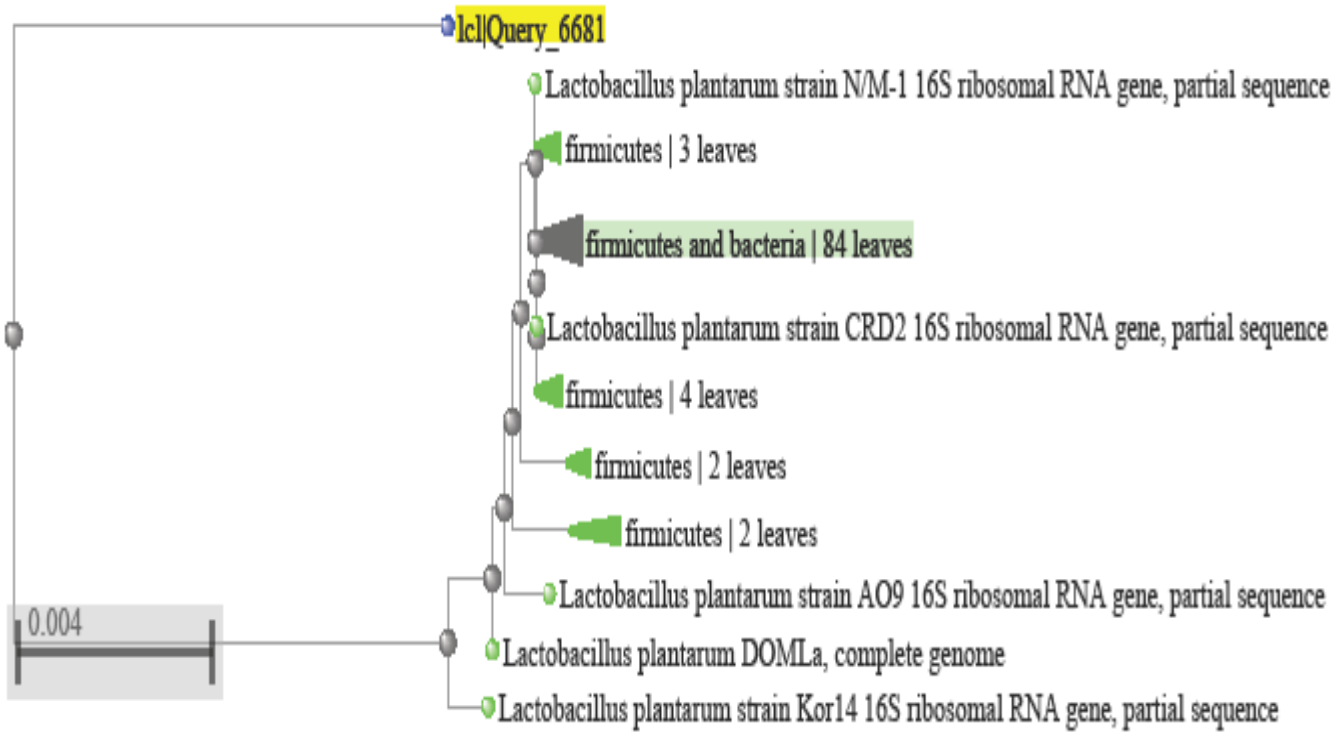
Fig .4 The *L. brevis* phylogenetic tree based on partial 16s rDNA sequences showing the relationship between isolate and other species belong to the genus *lactobacillus*

In the study, the 16S rRNA gene sequences were successfully amplified from 30 isolates by Polymerase Chain Reaction (PCR). The results showed a that the 16S rRNA gene sequences were successfully amplified with universal primers 27 F and 1492 R (Fig.2). Molecular characterization of the isolated strains were carried out based on 16S rRNA sequence analysis. The partial sequences of 16S rRNA obtained from isolates were aligned with all the presently available 16S rRNA sequences in the Gen Bank data base. The phylogenetic tree based on the partial 16s rDNA seqences showing the relationship between isolates and other species belonging to the geuns *Lactobacillus* as shown in figure 3, 4 and 5,

Showing 100% similarity towards *L . brevis* strain 100D, *L. plantarum* strains and *L. fermentum* strains. The phylogenetic analysis using 16s rRNA sequences indicated that the isolates belongs to the geuns *Lactobacillus* .The genomic diversity of lactic acid bacteria shows that different strains of LAB were present during fermentation and different LAB contributed to

the fermentation and flavor development. LAB will be highly relevant for the selection of LAB as starter cultures. Since the strains have long association with food consumed and will also be possible that some of its technological properties will be vital in biotechnological applications as reported by (Xiaodong, *et al*.1997).

Fig .5 The *L. plantarum* phylogenetic tree based on partial 16s rDNA sequences showing the relationship between isolate and other species belong to the genus *Lactobacillus*



Conclusion

A total of 30 LAB were successfully isolated from the newly bred yellow cassava dough and were identified by 16s rRNA gene sequencing and the 16s rRNA gene sequencing showed the lactic acid bacteria (LAB) as *L. brevis*, *L. fermentum* and *L.plantarum* with similarity index of 100%. The finding indicates that LAB from the newly bred yellow cassava dough can be used as probiotics and for food preservation.

Acknowledgement

The authors wish to acknowledge the National Root Crops Research Instutite Umudike, Nigeria for funding and International Institute of Tropical Agriculture,(IITA) Ibadan for sample preparation and laboratory facilities for the work.

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