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Isolation and Molecular Identification of Lactic Acid Bacteria from Some Fermented Foods

Uzoh C. V, Igwe P. C, Okeh C. Okeh, Aniokete U.C, Ugwu J.I, Yusuf I., Nworie C. O, Uzor S., Elom E.E, Umunna V.C

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ABSTRACT

Fermented foods serve as an important vehicle for microbial flora that form the human diet. Lactic acid bacteria are widely dispersed and implicated in almost every step of fermentation. The current study was done so as to isolate and identify lactic acid bacteria found in some locally fermented foods. Twenty (20) samples of fermented cassava and ogi were collected from different local food vendors. One gram (1g) each was dissolved in 9ml of distilled water, homogenized, plated out on MRS agar, and incubated at 37°C for 48 hrs. The isolates were identified and characterized by physiological and biochemical tests. Six (6) bacteria isolates were isolated and characterized. Species identification was based on the sequence analysis of 16S rRNA genes. The lactic acid bacteria species were found to be the predominant group of microorganisms involved in food fermentations. This has provided a baseline knowledge of the potential sources of probiotics and their application in functional foods.

Keywords Fermented foods, Lactic acid bacteria, Ogi, Cassava.

Uzoh C. V[1]*, Igwe P. C[2], Okeh C. Okeh[3], Aniokete U.C[4], Ugwu J.I[5], Yusuf I.[6], Nworie C. O[7], Uzor S.[8], Elom E.E[9], Umunna V.C[10]

[1,2,3,4,5,9]Lecturer 1, [6]Lecturer II

[1,3,6]Department of Microbiology, Alex-Ekwueme Federal University Ndufu-Alike Ikwo, Ebonyi State, Nigeria.

[2]Department of Microbiology, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria

[8]Senior Lecturer

[4,5,8]Department of Medical Laboratory Science, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria

[7] Lecturer, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.

[9]Department of Microbiology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

[10]GA, Department of Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State.

Email: optchuks@yahoo.com
*Corresponding author

INTRODUCTION

Advances in technology have continuously improved the quality of life of human beings. The connection existing between certain types of foods as well as their health advantages has been at the forefront of research. This made human beings realize that their diet is composed of mainly fermented food products of plant or animal origin. Fermented food products are now known to be consumed more globally than ever and constitute our major diet (Owusu-Kwarteng *et al*. 2015). With the quest of having consistent qual-

ity and improved safety of fermented foods, there is a need for the isolation of wild types of strains and the identification to the species level by employing molecular techniques. These lactic acid strains are used as functional starter cultures in the fermentation of food (Okorie *et al.* 2013, Owusu-Kwarteng *et al*. 2015). Recently, lactic acid bacteria have been the focus of research because of their highlighted importance in food fermentation, preservation, probiotics and functional foods. Cassava tubers can be processed into various African staple foods such as fufu and garri. It simply involves steeping roots in water until they soften or grating them. However, this takes about three to four days under optimal conditions to ferment (Ogbo 2013). Different microorganisms have been found to perform significant roles in the fermentation processes. In south-east Nigeria, ogi also called pap is a conventional fermented food that constitutes the major staple and weaning food. It is made from maize, guinea corn or sorghum. *S. cerevisiae, L. plantarum, Enterobacter cloacae,* and other lactic acid bacteria have been continuously isolated from fermented ogi (Egwim *et al*. 2013). The current study aims to isolate and characterize lactic acid bacteria from locally fermented foods using conservative as well as molecular strategies. The isolate could be used as a starter cultures in functional food production.

MATERIAL AND METHODS

Collection of samples

The collected samples were transferred to the Applied Microbiology Laboratory of Ebonyi State University for analysis with the aid of an ice-cubed box.

Isolation of Lactic Acid Bacteria from the Fermented Foods

Ten grams (10g) of each of the fermented cassava and Ogi were added to 90ml of distilled H_2O and homogenized for 5 minutes. After serial dilution, 0.1ml of the sample homogenate was plated out on De Man Rogosa sharp agar (Uzoh *et al.* 2022) and incubated anaerobically at 37^{o}C for 48 hours.

Identification of Lactic Acid Bacteria from Fermented Foods

Phenotypic Identification

Gram staining and other phenotypic characterization were conducted according to (Bansal *et al*. 2013).

Biochemical Characterization of the isolates

Catalase, oxidase, methyl red, Voges Proskauer, motility, indole, citrate and carbohydrate fermentation tests were conducted according to (Cheesbrough 2006, Mohammed 2018).

Catalase test

A grease – free slide was placed with one drop of three percent H_2O_2 and a loopful of the culture was emulsified on the slide. Bubbling was observed and noted (Cheesbough 2006).

Oxidase test

A few drops of oxidase reagent were added to the whatman number one filter paper and a 48h culture of the isolates was smeared on the filter paper with the aid of a wire loop. A color change was observed (Oyeleke and Manga 2008).

Motility test

After the growth of the organism on MRS broth at 37^{o}C for 24 hrs. A few drops of broth were placed on a fat-free glass slide and examined under an oil immersion (x100) lens for motility of the organism (Cheesbrough 2006).

Indole test

Lactic acid bacteria isolates were incubated in peptone water at 37^{o}C for 24 hrs. Then 0.5ml of kovac's reagent was added, homogenized and observed for color change (Mohammed 2018).

Methyl red test

Five milliliters (5ml) of glucose phosphate peptone was placed in a test tube. Each tube was individually inoculated with test cultures and incubated at 37^{o}C for 48 h. A few drops of methyl red solution were added

and a color change was observed after incubation (Mohammed 2018).

Voges Proskauer (vp test)

A sterile test tube containing 5ml of VP reagent with the organism was incubated at 37°C for 48 hrs. Five (5) drops of forty percent K_2O_2 and 15 drops of naphthol in ethanol were added and gently mixed. It was kept at a sloping position and the color change was observed (Oyeleke and Manga 2008).

Citrate Utilization Test

A clean test tube containing 5ml of Simon's citrate agar in slant form containing the isolates was incubated for 48 hrs and the color change was observed (Oyeleke and Manga 2008).

Carbohydrate Fermentation

Four milliliters (4ml) of pre-sterilized basal medium containing phenol red are inoculated with one percent (1%) of 6 sugars (lactose, ribose, glucose, mannitol, sucrose, maltose and fructose) and the organism. After incubation for 48 hrs, fermentation was observed (Oyeleke and Manga 2008).

Molecular Characterization of the Isolates

The DNA was extracted with a DNA purification kit (Zymo Research, USA) according to the instructions of the manufacturer.

DNA Amplification and 16srRNA Characterization

A pair of universal primers with forward primer (27F: AGAGTTTGATCMTGGCTCAG) and reverse primer (1525R: AAGGAGGTGWTCCARCCGCA) were used. PCR was carried out in a Gene Amp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with an initial denaturation at 94°C for 5 min; followed by 30 cycles consisting of 94°C for 30 s, 30 secs annealing of primer at 56°C and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10 mins and was maintained at 4°C (Agaliya and Jeevaratnam 2013).

Gel Electrophoresis of the DNA Fragments

Amplified products were separated by agarose gel electrophoresis using 1% agarose in 1X TBE buffer, 0.5µg/ml of ethidium bromide, and loading buffer (0.25% Bromophenol Blue in 40% sucrose). 5 µl of loading dye was added to 10 µl of PCR product and loaded on an agarose gel. Electrophoresis was performed at 90V for 55 minutes. The gel was viewed under UV light using a UV light transilluminator (Agaliya and Jeevaratnam 2013).

Sequencing and BLAST of Lactic acid bacteria isolates

The fragments were sequenced with Genetic Analyzer 3130 xl sequencer in accordance with the manufacturer's manual. The sequencing kit (BigDye Terminator version 3.1) was from Inqaba Biotech, SA. The software (Bio- Edit software and MEGA 6) was used while the sequence was blasted and compared with the sequences in NCBI in accordance with the procedures of (Endres *et al*. 2021).

RESULTS AND DISCUSSION

The result of the molecular characterization highlighted *Lactobacillus* sp as the organism with the highest occurrence. These organisms were seen to be existent and survive mostly in different habitats comprising plants, dairy, and fermented foods (Uzoh *et al*. 2022). In this study, different strains of *Lactobacillus* species were isolated from the different fermented foods. This result corroborates the work of (Ohenhen *et al*. 2015) who isolated 5 different *Lactobacillus* species from fermented samples of ogi. *Lactobacillus* species have been reported to be predominant in fermented foods. In this research, *L. plantarum* was the predominant species of lactic acid bacteria in the fermented cassava. This corroborates the study of Bansal *et al*. 2013 who reported a high occurrence rate of *L. plantarum* isolated from plant sources through fermentation. *L. pentosus* and *L. paracasei* were isolated from ogi (Samal *et al.* 2021). This contradicts the work of Egwim *et al*. 2013 who reported the presence of *L. plantarum* only in cereals. These isolates were phenotypically characterized generally as *Lactobacillus* species based on morphology and biochemical tests (table

Table 1. Physiological, morphological, and biochemical characteristics of the isolates.

Morphology		Gr	Mt	Physiological and biochemical characteristics													
				Cat-alase test	Oxi-dase	In-dole	Sugar fermentation test							Fruc-tose	Malt-ose	Suspected
Shape	Color						Methyl red	Voges proskau-er	Lac-tose	Ri-bose	Glu-cose	Man-nitol	Su-crose	+	+	Organisms
Rod	Round,-flat,smooth	+	-	-	-	-	-	-	+	+	+	+	+	+	+	*Lactobacillus* isolate 17
Cocci	Round Creamy colonies	+	-	-	-	-	-	-	+	+	+	+	+	+	+	*Lactobacillus* isolate 10
Rod	Irregu-lar,round,raised	+	-	-	-	-	-	-	+	+	+	+	+	+	+	*Lactobacillus* isolate 3
Bacilli	Mucoid, creamy	+	-	-	-	-	-	-	+	+	+	+	+	+	+	*Lactobacillus* isolate 11
Cocci bacilli	Round,raised, creamy	+	-	-	-	-	-	-	+	+	+	+	+	+	+	*Lactobacillus* isolate 7
Rod	Round, mucoid	+	-	-	-	-	-	-	+	+	+	+	+	+	+	*Lactobacillus* isolate 6

+ = positive, - = Negative, Gr = Gram reaction, Mt = Motility test.

1). Therefore, PCR amplification should be applied to further characterize the isolates into subspecies. *L. plantarum* and *L. pentosus* co-existed in the fermented cassava while *L. pentosus* and *L. paracasei* co-existed in Ogi. Their co-existence could be attributed to their competence in simple sugar utilization. This is in tandem with the work of David *et al*. 2019 who reported the possession of sugar utilizing cassettes in the lactic acid bacteria isolated. The 16S rRNA was amplified at about 1500bp using a 1kb DNA ladder (Fig. 1). The ancestry of the tree was divided into two major groups A and B. Group A was further sub-divided into two major groups division 1 and division

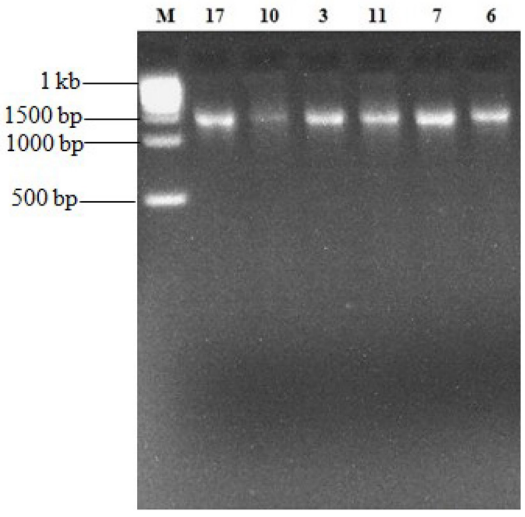

Fig 1. Gel electrophoregram of the lactic acid bacteria. M- standard DNA ladder, 17-*Lactobacillus plantarum* PRK7, 10- *Lactobacillus plantarum* Gt2, 3- *L. pentosus* MF19,7- *L. pentosus* MF19, 11- L. fermentum VDO3 and 6- *L. paracasei* TUTK20.

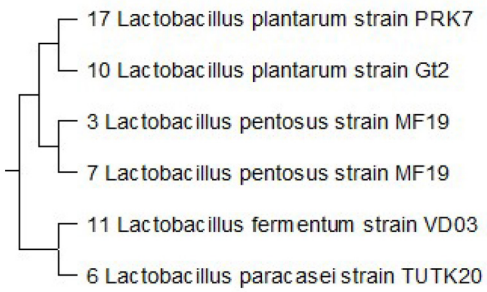

Fig 2. A phylogenetic tree of the 16SrRNA sequence of lactic acid bacteria.

2 (Fig. 2). Division 1 consists of isolates 17 and 10 while Division 2 consist of isolates 3 and 7. Group B consisted of isolates 11 and 6. Isolates 17 and 10 have a closer evolutionary relationship but are more diverse from isolates 11 and 6.

CONCLUSION

Lactic acid bacteria have been the predominant group of microorganisms present in fermented foods. They have been used in different food fermentations and have been used as sources of probiotics and for functional food production. The lactic acid bacteria have continued to be used in various commercial applications and biopreservation of foods. All the isolated bacteria in this study were specifically characterized by employing molecular techniques.

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