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Analysis of bacterial diversity during the retting of cassava for *fufu* production

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Abstract

Cassava (*Manihot esculanta*) is an important root crop that significantly contributes to food security, especially in Africa. Cassava is processed into edible foods such as fufu (a submerged fermented gruel). Fufu is a staple food in Eastern and Western Africa. Despite its widespread consumption and acceptability, there is a paucity of data on the diversity of the microbiota involved in fermentation (retting). The present study determined the microbial diversity of cassava retting for fufu production in laboratory-prepared samples. A total of eight bacteria species comprising *Enterobacter asburiae, Providencia vermicola, Klebsiella pneumonia, Citrobacter sp, Escherichia coli, Lactobacillus fermentum, Lactobacillus acidophilus* and *Lactobacillus plantarum* were implicated. pH decreased from 5.8 to 3.0 while temperature increased from 35 – 45 ^{0}C. *Lactobacillus plantarum* and *Lactobacillus fermentum* dominated the fermentation process. There's a need for a more robust study to characterize microbial communities during fufu production to determine functional species that could improve quality and ensure safety.

Keywords: Analysis; Cassava; Bacteria; Diversity; Retting; Fufu; Lactobacillus

1. Introduction

Cassava (*Manihot esculanta*) is an important root crop that significantly contributes to food security, especially in Africa. Cassava is processed into edible foods such as fufu (a submerged fermented gruel). Fufu is a staple food in Eastern and Western Africa. Despite its widespread consumption and acceptability, there is a paucity of data on the diversity of the microbiota involved in fermentation (retting). The present study determined the microbial diversity of cassava retting for fufu production in laboratory-prepared samples. A total of eight bacteria species comprising *Enterobacter sp, Providencia vermicola, Klebsiella pneumonia, Citrobacter sp, Escherichia coli, Lactobacillus fermentum, Lactobacillus acidophilus* and *Lactobacillus plantarum* were implicated. pH decreased from 5.8 to 3.0 while temperature increased from 35–45^{0}C. *Lactobacillus plantarum and Lactobacillus fermentum* dominated the fermentation process. There's a need for a more robust study to characterize microbial communities during fufu production to determine functional species that could improve quality and ensure safety.

Cassava is a food crop that significantly contributes to households' daily calorie intake, especially in Africa. Cassava is easily adaptable to poor soils with marginal nutritional status and pH ranging from 4 to 9. (1). Cassava thrives in suboptimal conditions. it is resistant to soil infertility, drought stress, and the majority of pests and diseases (2) and can be stored underground for several months after maturation (3). Cassava is processed into edible products by fermentation (such as *fufu*, *abacha*, *garri*) before consumption (4, 5, 6, 7, 8). Cassava-based fermented products are popular and are widely consumed by many people in East and West Africa.

Fufu is a starchy mash produced by the spontaneous fermentation of cassava. In the processing stages, freshly harvested cassava tubers are peeled, washed, sliced into small sizes and allowed to undergo spontaneous fermentation for 3-4 days (retting). Subsequently pulping, screening, sedimentation, dewatering and then cooking follow. (9).

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During retting, cyanogenic chemicals are destroyed, taste compounds are elaborated, and the roots are softened during the process (10). Previous studies on the diversity of *fufu* have been based on the isolation and identification of microbiota from ready-to-eat fufu (11, 12). There are also reports on the targeted isolation of *Lactobacillus* from retted cassava (7,8,13). There is a paucity of data on microbial diversity during cassava's retting for fufu production. In view of the wide consumption of *fufu*, such a study is needed to (i) evaluate potential pathogens that could pose a food safety risk, and (ii) provide diversity data useful in identifying starter culture candidates and safe food production of fufu. This work was therefore designed to analyze the bacteria diversity during the retting of cassava for fufu production.

2. Material and methods

2.1. Sample collection

Cassava tubers were harvested from a farm in Egbu, Owerri North, Imo State, and were transferred into a sterile polyethene bag and immediately delivered to the laboratory for analysis.

2.2. Retting of fufu in the laboratory

The cassava tubers were washed with sterile distilled water and cut into slices. It was submerged into a sterile bowl containing 1000 mls of sterile distilled water at an ambient temperature (30^0C). Samples of cassava retting water were collected for microbial enumeration after 72 hrs. The temperature and pH of the water was monitored at 24 hrs interval

2.3. Isolation and Enumeration of bacteria from food samples

Serial dilutions of each sample were carried out using 0.1% peptone water. Aliquots of 0.1ml were pour-plated on standard plate count agar (Oxoid, UK), MRS agar (Oxoid) and MacConkey agar (Oxoid, UK). Plates were incubated at 37°C for 24 hours and counted using the colony counter. MRS plates were incubated anaerobically for 48 hrs. Only colonies growing on plates containing between two to ten colonies were sampled. Isolates were purified by streaking on plate count, MRS and MacConkey agar plates.

2.4. Identification of isolates

The isolates were identified based on their cell morphology. Biochemical tests such as catalase, oxidase, motility, citrate utilization, vogues Proskauer, methyl red, indole, and carbohydrate utilization were also done. Gram-stained smears of the isolates were viewed with a phase-contrast microscope (Olympus, Tokyo, Japan). *Lactic acid bacteria were* confirmed by using the standard commercial identification system API20 CHL (Biomerieux®, France), according to the manufacturer's instructions. Pure cultures of lactic acid bacteria were maintained on MRS slants while other isolates were maintained on standard plate count agar.

3. Results and discussion

Table 1 Morphological and biochemical characteristics of bacteria isolates

Cell shape	Gram reaction	Catalase	Citrate	Oxidase	Indole	H_2S	Gas	Lactose	Sucrose	V.P	M.R	Glucose	Maltose	Sorbitol	Motility	Urease	Probable organisms
R	-	+	+	-	-	-	+	-	+	+	-	+	+	+	+	_	*Enterobacter sp*
R	-	+	-	-	+	-	+	+	+	-	+	+	+	+	+	-	*Escherichia coli*
R	-	+	-	-	+	-	X	X	X	-	+	+	-	+	-	+	*Providencia vermicolar*
R	-	+	+	-	+	-	+	+	+	+	-	-	+	+	-	+	*Klebsiella pneumonia*
R	-	+	+	-	+	-	+	+	X	-	+	+	X	+	+	X	*Citrobacter sp*

Keynote: + = positive, - = negative, x = not carried out

A total of five bacteria genera belonging to *Enterobacter sp*, *Escherichia coli*, *Citrobacter* sp, *Providencia vermicular* and *Klebsiella pneumonia* (Table1) while *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Lactobacillus plantanrum* were identified using the analytic profile index (API 50 CHL) from the fermented cassava gruels (Table 2).

Table 2 Biochemical Characteristics of lactic acid bacteria using API-50CH

Gram Stain	+	+	+
Catalase	-	-	-
Glycerol	-	-	-
Erythritol	+	-	-
D-Arabinos	-	-	-
L-Arabinose	-	+	-
Ribosa	+	+	-
D-xylose	+	+	-
L-xylose	+	-	-
Adonitol	-	-	-
β–Μετλλ–Δ–Ξιλοσιδε	-	-	-
Galactose	+	+	-
D-Glucose	+	+	+
D-Fructose	+	+	+
D-Mannose	+	-	+
L-Sorboso	+	-	-
Rhamnose	-	-	-
Dulcitol	-	-	-
Inostitol	-	-	-
Mannitol	+	-	-
Sarbitol	+	-	-
α-methyl-D-mannoside	+	-	-
α-methyl-Glucoside	+	-	-
Amvadalin	+	-	-
Arbutin	+	-	-
Esculin	+	-	+
Salicin	+	-	-
Cellobiose	+	-	+
Maltose	+	-	-
Lactose	+	-	-
Melibiose	+	-	-
Saccharose	+	+	+
Trehalose	+	-	-
Insulin	+	-	-

Melezitose	-	-	-
D-Raffinose	+	-	-
Amidon	+	-	-
Glycogen	-	-	-
Xylitol	-	-	-
β–gentibiose	-	-	-
D-Turanose	+	-	-
D-lyxose	+	-	-
D-Tagatose	-	-	-
D-Fucose	+	-	-
L-fucose	-	-	-
D-Arabitol	-	-	-
L-Arabitol	+	-	-
Gluconote	-	-	-
2-keto-gluconate	+	-	-
5-keto-gluconate	-	-	-
HM/HE	HM	HE	HM
Growth at 4% NaCl	+	+	+
Growth at 15^{o}c	+	+	+
Growth at 45^{o}c	-	-	-
Species identified	L.P	L.F	L.A

Key: + = Positive, - = Negative, HM = Homofermenter, HE = Heterofermenter, L.P = *Lactobacillus plantarum*, L.F = *Lactobacillus fermentum*, L.A = *Lactobacillus acidophilus*

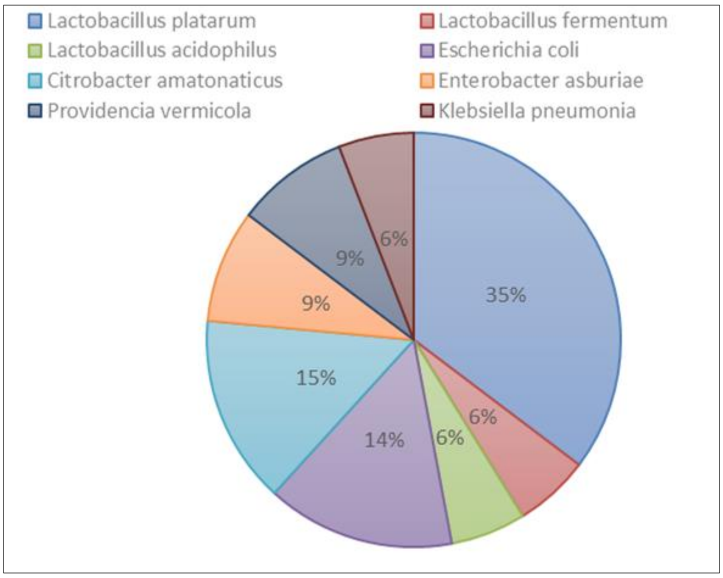

Figure 1 Percentage occurrence of bacteria isolates from retted cassava samples

Previous reports of bacterial diversity in fufu have also implicated *Enterobacter*, *Klebsiella* and *Lactobacillus* (4, 10,13,14, 15) The presence of *E. coli* and *Providencia* may suggest contamination of cassava tuber directly from the soil. According to 16 and 17, the presence of *Providencia sp* may also have resulted from poultry feaces deposited in the farm.

The presence of *Providencia vermicolor* is a public health concern since some species can potentially cause infection in humans. *E. coli* has been documented to be responsible for diarrhea in humans (18). *Enterobacter sp* is an opportunistic pathogen and does not pose a significant threat to humans. *Lactobacillus plantarum* was the most predominant isolate (Fig 1).

Numerous studies have reported *Lactobacillus plantarum* as the predominant organism from fermented cassava mash. (7, 19, 20, 21, 22, 23), *L. plantarum* and *L. fermentum* have been implicated as the predominant microorganism in most fermented food especially carbohydrate based. The increase in pH (Fig 2) observed throughout the fermentation could be attributed to the activities of lactic acid bacteria. This trend has also been reported by several authors (23, 24, 25, 26,).

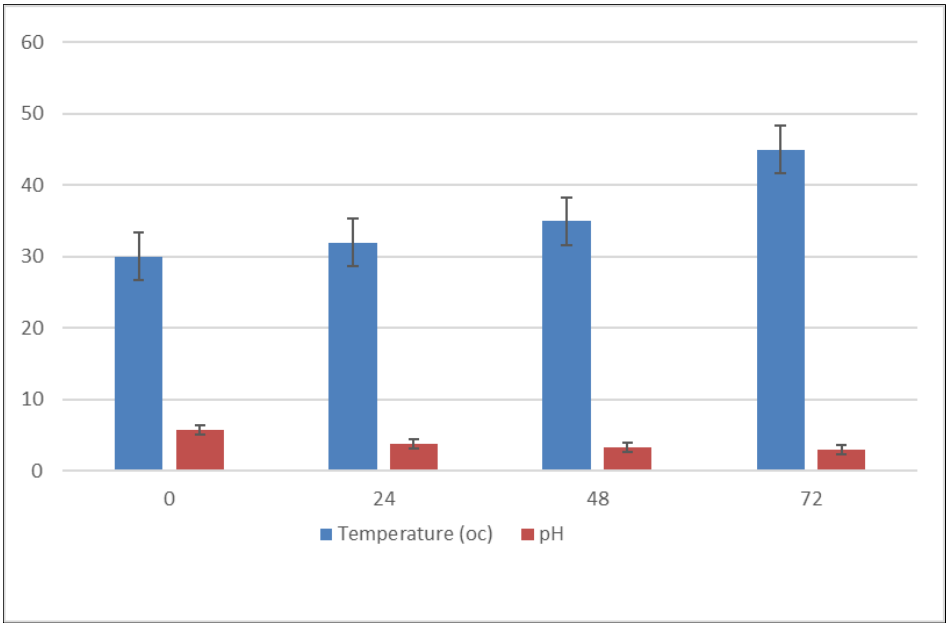

Figure 2 Physicochemical changes during retting

4. Conclusion

The present study determined the microbial diversity of cassava retting for fufu production in laboratory-prepared samples. A total of eight bacteria species comprising *Enterobacter asburiae, Providencia vermicola, Klebsiella pneumonia, Citrobacter sp, Escherichia coli, Lactobacillus fermentum, Lactobacillus acidophilus* and *Lactobacillus plantarum* were implicated. *Lactobacillus plantarum* and *Lactobacillus fermentum* dominated the fermentation process. However, there is need for a more detailed study on microbial diversity and succession dynamics during fufu production in order to improve food quality and safety.

Compliance with ethical standard

Acknowledgement

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Disclosure of conflict of interest

No conflict of interest exists.

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