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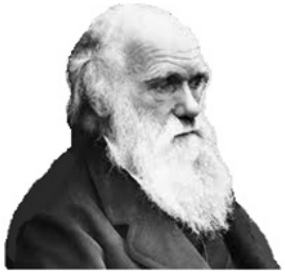

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Microorganism Associated with Cassava Fermentation for Abacha Product Sold in Owerri, Imo State

Francis Chukwuebuka Ihenetu[1,*], Stanley Chukwuemeka Ihenetu[2], Chinyere N. Ohalete[3], Treasure Njideka Njoku-Obi[3], and **Bright Chinemerem Okereke[1]**

[1]Department of Microbiology, Federal University of Technology Owerri, Nigeria

[2]Department of Chemistry, Imo State University, Owerri, Nigeria

[3]Department of Microbiology, Imo State University, Owerri, Nigeria

*E-mail address: ihenetufrancis@gmail.com

ABSTRACT

The microorganism associated with cassava fermentation for Abacha product was investigated using four different Farms in Owerri (Obinze, Umugwuma, Urata and Orji) as sample collection area. Four of the cassava tubers harvested, washed, cooked and soaked with water after cutting to produce Abacha then was fermented using standard microbiological methods. The waste water was sampled and all the samples A, B, C, and D showed significant bacteria growth using NA, MRS, PDA. In the study, six bacteria and fungi isolates identified were *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus* spp, *Candida* spp., *Aspergillus* spp. and *Bacillus* spp. Results showed that the total heterotrophic bacteria count of the sample varies within 8.0×10^{10} to 2.5×10^{12} and total fungi count (TFC) ranges from 6.6×10^{9} to 9.6×10^{9}. The study also showed that *Lactobacillus* spp. and *Escherichia coli* had the highest occurrence of 75.00% ($P < 0.05$) prevalence occurrence while *Staphylococcus aureus*, *Saccharomyces* spp., *Bacillus* spp. and *Candida* spp. had a percentage occurrence of 50.00% respectively. There is therefore urgent need for public enlightenment on public health implications, need for proper hygiene as well as strategies for preventing and controlling the microorganism which do not play a role in the fermentation of cassava for Abacha production.

***Keywords*:** Abacha, Cassava, Microorganism, Fermentation, Bacteria and fermentation

1. INTRODUCTION

Cassava, a relatively unknown crop in the old world before the discovery of America is fast assuming the status of the saviour of the world, as it is second to potato as the most important starchy root crop of the tropics used for food and industrial purposes [1]. It is an important economic crop cultivated in many tropical countries of Asia, South America and Africa where it provides calories for millions of people [2]. Its capacity to survive and even thrive in adverse conditions and high yield potential has endeared it to many farmers. It is a major staple food crop in Nigeria and supplies *about* 70% of daily caloric intake of 50 million Nigerians. Nigeria is the largest world producer of cassava with over 34 metric tons of the crop produced annually [3,4].

Abacha is a dry or wet product, obtained by shredding or slicing boiled cassava tubers, soaking the shreds for 8 – 24 h in cold water, washing and drying if need be. It is consumed as a snack food or main meal in the Eastern States of Nigeria [5]. One of the major constraints in the utilization of cassava is its poor storage potential [6]. Cassava like most agricultural produce has its peak time when it yields optimum product. The inability to process the entire root harvested due to inadequacy of processing facilities and short storage period of the cassava leads to spoilage and food losses. The post-harvest losses of fresh cassava could be as high as 23% [8].

Another factor constraining the output of root is the difficulty in extracting it from the hard ground during the dry season which results in artificial scarcity of cassava products like *gari* [9].

2. MATERIALS AND METHOD

Cassava sample

Different cassava tubers were harvested in four different farms in Owerri, Imo State which include; Obinze, Umugwuma, Urata and Orji labelled A, B, C, and D respectively.

Laboratory analysis

The freshly harvested cassava roots were manually peeled with stainless steel knife, washed and cut into chunks (7.0-8.0 cm length) and boiled in water for 20 min. The cooked samples were cooled, thinly sliced (0.50-0.80 mm thick) and soaked for 16 h. During the soaking, the soak water was routinely changed after 4 h. The slices were properly washed to obtain fresh wet ‘abacha’ slices. The waste water was used for ten (10) fold serial dilutions and spread plate method was adopted [10].

Cultural Analysis

Stock cultures of isolates were maintained on nutrient agar slant for further bacteria identification and the fungi identification was also determined using standard microbiological methods [12].

Identification of Fungi and Bacterial Isolates

The fungi isolates were identified using colonial and cellular characteristics, and bacterial were identified using colonial, cellular characteristics, and biochemical properties. Biochemical tests carried out include; Urease test, Citrate utilization test, Indole test, voges-proskauer test, Motility test, Methyl-red test, Coagulase test, Sugar fermentation test and Catalase test. [13]

Total Heterotrophic Count

From each dilution, 0.1 ml was collected and dispensed aseptically into fresh sterile nutrient agar duplicates and then spread with a sterile bent glass rod. The inoculated plates were incubated for 24 hours at 37 °C. The discrete colonies were counted and sub cultured onto fresh nutrient agar plate followed by incubation at 37 °C for 24hours. They were then transferred into nutrient agar slants and stored in the fridge for biochemical analysis.

Total Fungi Count

The inoculated PDA plates were incubated for at 37 °C for 3-5days and identified using their cultural and morphological characteristics.

3. RESULTS

Overall Growth of Microorganism in the Study

In the study, a total of four (4) samples were examined for microorganism. It was observed that all of the samples examined where had either bacteria or fungi growth. The characterization of the different microbial isolates is shown in the Table 1 and 2 below.

Table 1. Morphological and Biochemical Characteristics of the Bacterial isolates

COLONY/ MORPHOLOGY	Gram stain	Spore	Motility	Urease	Catalase	Citrate	MR	VP	Indole	H_2S	Coagulase	Glucose	Lactose	Sucrose	Probable organisms
Gray to white on MSR.	+ve rods	+	+	+	+	+	+	-	-	-	-	AG	-	-	*Bacillus* spp.

Cream, smooth, raised, circular.	+ve coci in clusters	-	-	-	+	+	-	-	-	-	+	A	-	-	*Staphylococcus aureus*
Cream white non-viscous flat.	-ve shot rods	-	-	-	-	-	-	-	+	-	-	A	A	A	*Escherichia coli*
Cream, rough, opaque and circular	+ long, rods in chains	+	-	-	-										*Lactobacillus* spp.

KEY: + : positive; -: negative; A: acidic; G: gas

Table 2. Morphology properties of the fungi isolates.

CULTURE CHARACTERISTICS	CELL MORPHOLOGY	PROBABLE ORGANISMS
Cream white smooth and flat	Oval budding cells, pseudo-hyphae	*Candida* spp.
Whitish with yellow Reverse (for young culture) Blue-green to dark-green (for old culture)	Double branching septate hyphae, short conidiophores	*Aspergillus* sp.

Identification of Microbial Isolates

Four bacteria genera were isolated then characterized to belong to the genus *Bacillus* sp., *Escherichia coli*, *Staphylococcus aureus*, and *Lactobacillus* spp. and two Fungi genera were then characterized to belong to the genus *Candida* spp. and *Aspergillus* spp.

The results of the identification of bacteria isolates are shown in Table 3.

Table 3. Microorganisms isolated from samples from different sampling.

Samples	Organisms
A	*Lactobacillus spp., Staphylococcus aureus., Escherichia coli, Saccharomyces* spp.
B	*Bacillus cereus; Escherichia coli, Saccharomyces* spp., *Lactobacillus* spp.
C	*Lactobacillus* spp., *Bacillus spp. Candida* spp.
D	*Staphylococci* spp., *Escherichia coli; Candida* spp.

Total Heterotrophic and Fungi Count on the Isolates

Table 4 shows the microbial load of the fermented cassava for Abacha samples shows the total heterotrophic bacteria count and the total Fungi count. The total heterotrophic bacteria (THBC) and Total fungi count of the sample A, B, C and D is shown in the table below respectively.

Table 4. Total Heterotrophic and Fungi Count On the isolates

SAMPLES	TOTAL HETEROTROPHIC BACTERIA COUNT (Cfu/g)	Total fungi count (Cfu/g)
A	2.5×10^{12}	9.6×10^{9}
B	4.55×10^{11}	3.8×10^{10}
C	4.0×10^{11}	6.6×10^{9}
D	8.0×10^{10}	9.6×10^{9}

Percentage Occurrence of Bacteria and Fungi Isolates

Presented in Tables 3 is the occurrence of bacteria and fungi isolated from the samples from the result, samples bacteria and fungi growth was checked for their occurrence prevalence *Lactobacillus* spp. and *Escherichia coli* had the highest occurrence of 75.00% prevalence while *Staphylococcus aureus*, *Saccharomyces* spp., *Bacillus* spp. and *Candida* spp. had a percentage occurrence of 50.00% respectively. The result is shown in Figure 1

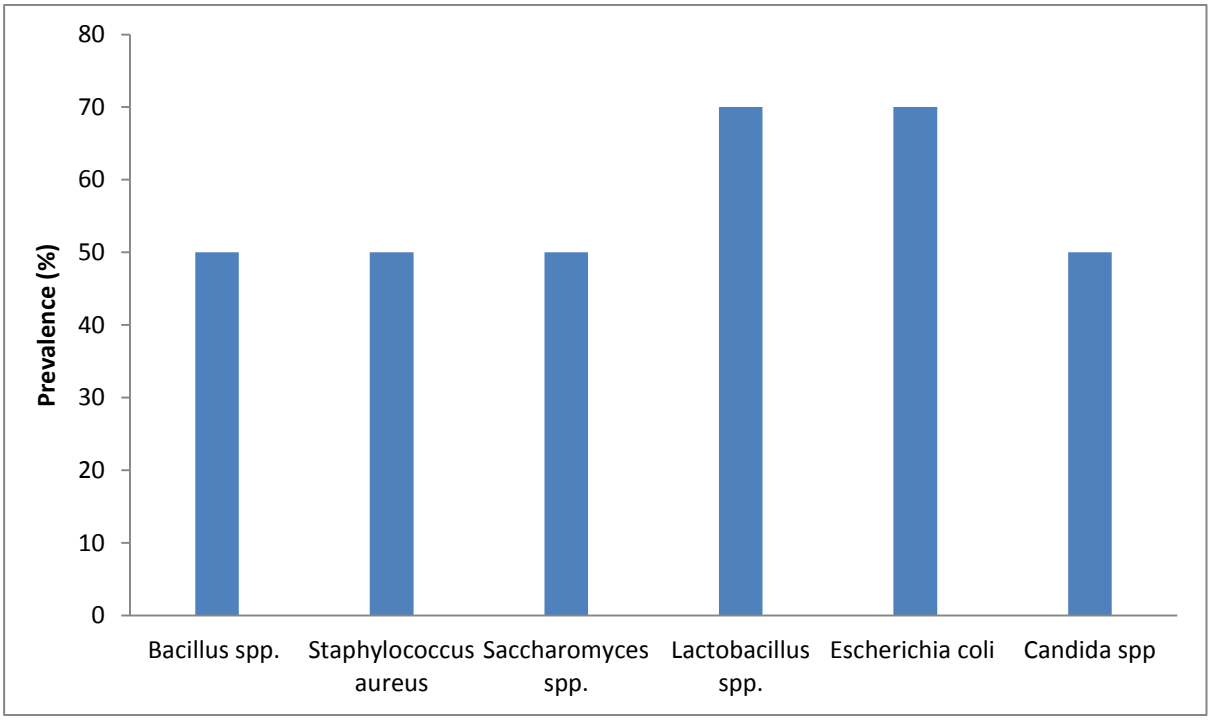

Fig. 1. Percentage occurrence of bacteria and fungi isolates

4. DISCUSSION

After series of microbial examination ranging from total Heterotrophic bacteria Counts and fungi count, spore stain and gram staining to biochemical test, results obtained showed the presence of bacteria and fungi in the fermented cassava for Abacha production was obtained. Of the four fermented cassava for Abacha production samples examined all the samples contains both bacteria and fungi growth. The organism that was isolated on whole include; *Bacillus sp., Escherichia coli, Staphylococcus aureus, Lactobacillus* spp., *Candida* spp. and *Aspergillus* spp. This finding agrees on the report of Perrine [14] who reported that bacteria growths occur in cut of the tubers and some reservoirs and improper washing of the tubers. The result suggested different cases or sources of contamination of the cassava samples. The findings also showed the percentage prevalence ($P < 0.05$) of the bacteria and fungi agents which showed that *Lactobacillus* spp. and *Escherichia coli* had the highest percentage prevalence of occurrence of 70.00% ($P < 0.05$) (Fig: 1). The various bacterial isolates obtained from the cassava waste water have also been isolated from fermenting cassava by various workers [15]. Also it was observed in the study that Retting of cassava tubers allows softening of the roots for further processing and the reduction of the potentially toxic cyanogenic glycosides present in the roots. Many microbes had been reported to be responsible for cassava fermentation to produce Abacha. It was found that some of these organisms in the system are not useful for retting to occur. There is therefore need to eliminate the unwanted organisms and use the useful starter cultures [16,17].

5. CONCLUSION

The dictation and isolation of bacteria and fungi from apparently fermented cassava for Abacha production shows that there was growth of some organisms that are pathogenic to human. Therefore adequate care should be taken when washing, fermenting, processing and storing them.

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