

IDENTIFICATION OF INDIGENOUS BACTERIA FROM SOIL CONTAMINATED WITH CASSAVA MILL EFFLUENTS

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ABSTRACT

This study was to isolate and identify the bacteria in soil contaminated with cassava mill effluent in Ifite Awka, Anambra State, Nigeria. Soil samples from two different locations polluted with cassava mill effluent were collected. The isolation and identification were carried out using standard analytical methods. The agars used were Nutrient agar and MacConkey agar. The population of bacteria (microbial count) was determined. *Pseudomonas* spp., *Staphylococcus aureus*, *Lactobacillus* spp., and *Bacillus* spp. were isolated from the soil samples and characterized. The microscopic analysis showed that all the bacteria isolates were gram positive except one. *Bacillus* spp. had the highest frequency of occurrence while *Lactobacillus* spp. had the least frequency of occurrence. Soil contaminated with cassava mill effluent caused some physicochemical changes in the samples collected which were: Cyanide content 3.0 mg/kg, conductivity 33.4µS/cm, phosphate 0.52 mg/kg, nitrate 0.35 mg/kg, sulphate 13.0 mg/kg, calcium 167.0 mg/kg, pH 6.3 mg/kg, magnesium 89.0 mg/kg, potassium 4.0 mg/kg and sodium 92.0 mg/kg. This study thus reveals that the cassava mill effluent has some deleterious effects on soil structure, soil microbiota, and soil quality, and hence recommends that appropriate measures be put in place to enforce and regulate the treatment of such effluents prior to discharge to receiving soil. The cassava effluent should therefore be treated before discharge into the environment to prevent possible pollution.

Keywords: *Manihot esculenta* Crantz, Contamination, Effluents, Mill, Bacteria

1. INTRODUCTION

Root and tuber crops are grown for their starch content, and are important staples of tropical Africa (Frank and Kingsley, 2013; Agu et al., 2014; Okigbo et al., 2015; Agu et al., 2016; Agu et al., 2016). The genus *Manihot* incorporates over 200 species of which *Manihot esculentum* Crantz is the most important, from the nutritional and economic point of view commonly known as cassava, manioc, cassava and yucca cassava (*Manihot esculenta* Crantz) is a root tuber crop that is widely cultivated in the tropical regions of the world (Oboh and Akindahunsi, 2013). It is mainly a food crop whose tubers are harvested between 7-13 months based on the cultivars planted the tubers are quite rich in carbohydrate (85.9%) with very small amount of protein (1.3%) in addition to cyanogenic glycoside (Nwabueze and Odunsi, 2010). This high carbohydrate content makes cassava a major food item especially for the low income earners in most tropical countries especially Africa and Asia (Desse and Taye 2016).

Cassava is believed to have originated from South America to other Northern America, cassava was introduced in the 16th century around Congo river basins (Cock, 2002). In sub-Saharan Africa, cassava is a major staple food that is consumed in processed forms in many areas. In West Africa and Nigeria in particular the crop is mostly consumed as garri, a dry granulated meal made from fermented cassava (IITA, 2001). Currently, Nigeria is the highest producer of root/tuber crops in the world with growth and processing of more cassava for domestic and international needs (Frank and Kingsley, 2013).

However if the contribution that cassava can make to the lives of poor people is to be increased, there is need to consider also its post-harvest handling, processing, and marketing. Both cassava roots and leaves can be used as food, but economically the roots are usually more important, although in some part of African countries, the leaves may be more important or more important than the roots. Cassava is one of the most important food crops in Africa (Agu et al., 2015). It derives its importance from the fact that its starch, thickened tuberous roots are valuable source of cheap calories especially in developing countries, caloric deficiency and malnutrition are wide spread. Over two thirds of the total production of cassava is consumed in various forms by humans, its usage as a source of ethanol for fuel, energy in animal feed and starch for industry increasing. The crop is amenable to agronomic as well as genetic improvement, has a high yield potential under good conditions and performs better than other crops under sub-optimal conditions. It is grown widely in several countries in sub-Saharan Africa and Madagascar the importance of cassava in food security and nutritional issues has led IITA (International Institute of Tropical Agriculture) and the

united nations children's education fund (UNICEF) to establish their joint household food security and nutrition programs with the goals of extending the benefits of IITA research to Africa countries through UNICEF's country programs of social mobilization development (Nweke, 2011). Africa as one of the largest produce worldwide produces over 50 million tones of cassava annually (FAO, 2001). Total world cassava use is expected to increase from 172.7 million tons to 275 million tons in the period of 1993-2020 using the international food policy. Research institutes (IFPRI's) base line data. As higher prediction of demand and production growth puts the 2020 production at 291million tons (Scott et al; 2005). Traditional garri production is associated with discharged of large amount of water, hydrocyanic acid and organic matter in the form of peels and sieves from the pulp as waste products. Around cassava mill, the liquid waste is indiscriminately discharged and allowed to accumulate, producing offensive odors and unsightly scenarios (FAO, 2004; Okafor, 2008; shiadgonareetal; 2009;). The high cyanide content from the effluent equally posses significant threat to humans and the environment, which calls for regulations in the discharge of the waste generated (Akani et al; 2016; Adewoye et al., 2015). Kolwan et al., 2016) defined soil as the top layer of the earth's lithosphere, formed from weathered rock that has been transformed by living organisms soil, formation is the result of the combination action of weathering and Colonization of geological material by microbes (Wiley et al.;2008). Soil also has many layers, with the topsoil being the most productive. The biological components of the topsoil consist mainly of soil organisms especially microorganisms which are key players in the cycling of nitrogen, sulphur and phosphorus and the decomposition of organic residues these affects nutrient and carbon cycling on global scale (Burning and Jimenez, 2003). The topsoil receives the greatest impact from pollutants. The effluents when incorporated into the soil exert effects on the soil itself. When discharged, it is acted upon by nutrients and soil microorganisms, releasing gases into the soil which other breakdown products are trapped in the soil (Pelczar et al; 1993).

2. STATEMENT OF THE PROBLEM

The increased utilization of processed cassava products has increased the environmental pollution associated with the disposal of effluents. The highly offensive odour emanating from the fermenting effluent calls for regulation in the discharge of waste generated. In most areas, cassava mills are mainly on small scale basis, owned and managed by individuals who have no basic knowledge of environmental protection. Though on small scale basis, there are many of them, which when put together, create enormous impact on the environment. Cassava also contains much pollutant such as disease-causing pathogens e.g. bacteria and fungi. Disposal of agricultural by-products such as cassava waste from processing activities is a concern in Nigeria.

There is an appreciable high level of contamination arising from the discharge of effluents on agricultural soil hence the need for proper treatment before discharge and conversion of these cassava wastes into biosorbent that can remove toxic and valuable metals from the effluent. Reports have shown that the cassava effluent contains harmful cyanides, copper, mercury and nickel which have the capacity to affect native micro-biota (Aiyegoro et al., 2017). Pollution from such effluent could result to a serious imbalance in the living and non-living entities of the ecosystem (Lemke et al., 2004) this could lead to a reduction in the soil fertility. Cassava mills, which are mainly on small scale platform, are processed and managed by persons who lack knowledge of environmental safety. While on a small scale platform, there are many of them, when combined together they produce great negative effects on the ecosystem (Nwaugo et al., 2018). Thus, it is essential to estimate the extent of such effects on both biotic and abiotic components of the ecosystem.

3. MATERIALS AND METHODS

Sample Collection: Two (2) different samples were collected from two different location in ifite, Awka. Spatula was used to collect soil samples into sterile containers. All samples were transported immediately to the laboratory for analysis after collection.

Sterilization of Materials: The sample bottles and other materials were thoroughly sterilized before use.

Preparation of Media: Nutrient Agar (NA): Dissolve 28.0g in 1 liter of purified water. Add one tablet of nystatin (properly grounded in aluminum foil), mix properly. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121°C) (Aryal, 2015).

Preparation of soil samples for microbiological test: 10grams of soil sample were weighed into 100mls of sterile water to be diluted then placed in laboratory shaker for the soil sample to dissolve properly in the sterile water.

Isolation of bacteria: Spread plate method was used. Approximately 2 or 3 drops of the serial diluted soil sample was placed on each type of media and incubated for 24hours at 37°C.

Subculture: The colonies from the plates were observed for morphological differences. Each colony that differed morphologically from another was picked with a sterilized wire loop and inoculated in a freshly prepared nutrient agar and incubated for 24hours at 37°C in order to obtain pure colonies of the isolates. After obtaining pure colonies, the colonies were inoculated in slant bottles and stored for further identification.

Characterization and identification of the isolates: Isolates were characterized using routine microbiological procedures. The microbiological identification procedures used include:

- **Colony morphology:** This involves the microscopic evaluation of the characteristics of bacteria colonies on the agar plates. The characteristics considered include the shape of the colony, elevation of the colony, edge of the colony, colony surface pigmentation and the optical characteristics.

Identification and Confirmation of Isolates

Pure cultures were isolated. Their various cultural appearances were recorded, followed by microscopic and biochemical tests to identify the isolates. Microscopic and biochemical tests done using standard methods include; Gram staining, motility, catalase, citrate, oxidase, indole and hemolysis test.

- **Gram Staining**

A drop of Lugol's iodine was placed on a clean grease-free slide and a colony in isolates was picked with a sterilized wire loop and emulsified. The glass slide was passed over the flame three times to heat fix. The smear was flooded with crystal violet for 60seconds and rinsed with distilled water. Lugol's iodine was added and rinsed, then decolourized with acetone and rinsed immediately with distilled water. The smear was counter stained with Safranin for 1 to 2 minutes and rinsed with distilled water. The smear was then allowed to air dry after which oil immersion was added and viewed under microscope using x100 objective lens (Cheesbrough, 2010).

- **Catalase Test**

The container containing Hydrogen peroxide solution was shaken to expel the dissolved oxygen. One drop of the solution was dropped on a clean glass slide followed by the addition of a loopful 24hour old inoculum of the slide, presence of gas bubbles indicate a positive result while the absence of gas bubbles indicates negative result (Cheesbrough, 2010).

- **Simmon Citrate Test**

Simonm citrate agar was prepared and poured in a test tube to sterilize for about 1hour, 30min. The test tubes were slanted and allowed to gel. A wire loop was sterilized and used to collect from the pure culture and then smeared on the Simmon citrate agar. It was then incubated for 24hours at 37°C. A positive citrate was confirmed by formation of blue colour while the initial green colour denoted a negative result. Results were recorded (Cheesbrough, 2010).

- **Indole test**

Glucose phosphate peptone broth was prepared. A wire loop was sterilized and used to collect from the sub-culture bacteria and rinsed into the peptone water. This was then incubated for 24hours at 37°C. Kovard reagent was then added and observed for results. A red colour change in the first 10 seconds indicated a positive result, while no colour change indicated a negative result (Cheesbrough, 2010).

- **Motility test**

Nutrient agar was prepared. A wire loop was sterilized and used to collect from the sub-culture bacteria and then stabbed into the solidified agar and incubated for 24hours. Motility was observed by spread of organism outwards from the stab area (Cheesbrough, 2010).

- **Haemolysis test**

Nutrient agar was prepared and 10ml of blood added onto it and allowed to solidify forming blood agar. Sample organisms is inoculated into it and incubated.

- **Urease test**

Urea agar was prepared by dissolving 3.3g of the agar in 250ml of water and allowed to homogenize by heating using bunsen burner and dispensed in test tubes then stopped with cotton wool and autoclave, the test tubes were slanted and allowed to solidify, the isolates were inoculated into the test tubes by making streaks around the slanted area. The development of an intense magenta to bright pink colour within 24hours indicates positive but lack of colour change indicates negative. (Takahiro et al.,2018).

4. RESULTS

Bacteriological count of colonies present in soil sample- The soil sample were diluted and inoculated in Nutrient and MacConkey Agar then incubated in the incubator for 24hours. The results in table 1 below shows the bacteriological count of colonies present in the soil sample.

Table 1: Bacterial/Colony count from soil sample

Media	Sample	Dilution Factor	Growth/No growth	Colony count (cfu/g)
Nutrient Agar	A	10 ⁻⁵	Growth	3.55×10 ⁶
	A	10 ⁻⁶	Growth	1.43×10 ⁶
	B	10 ⁻⁵	Growth	4.56×10 ⁶
	B	10 ⁻⁶	Growth	1.82×10 ⁶
	Control sample	10 ⁻⁵	Growth	5.5×10 ⁶
		10 ⁻⁶	Growth	4.25×10 ⁶
MacConkey Agar	A	10 ⁻⁵	No growth	
	A	10 ⁻⁶	No growth	
	B	10 ⁻⁵		
	B	10 ⁻⁶	No growth	
	Control sample	10 ⁻⁵		
		10 ⁻⁶		

Morphological Characteristics of the Bacterial Isolates-From the various bacterial colonies, four (4) distinct colonies were selected for further identification. The morphological characteristics of the bacteria considered were the form, pigmentation, elevation, margin, and opacity. The results are shown in table 2 below:

Table 2: Morphological Characteristics of the Bacterial Isolates

Isolate	Form	Elevation	Pigmentation	Opacity	Margin
Colony A	Circular	Flat	Grey	Opaque	Entire
Colony B	Circular	Convex	Yellow	Opaque	Entire
Colony C	Irregular	Raised	White	Translucent	Entire
Colony D	Irregular	Slightly convex	Creamy	Opaque	Lobate

Microscopic and biochemical tests for identification of bacterial isolates-The microscopic analysis showed that all the bacterial isolates were gram positive except one. They consist of cocci, short rods, and long rods. The various biochemical tests carried out were catalase, motility, urease, Simmon citrate, Indole, and Hemolysis. The results of the microscopic and biochemical tests are displayed in table 3 below;

Table 3: Biochemical Test of the Bacteria Isolates

Isolate	Gram stain	Microscopy	Motility test	Catalase test	Hemolysis test	Citrate test	Indolase test	Urease test	Probable organisms
1	-ve (Pink colour d rods)	Rod	+ve	+ve	+ve	+ve	-ve	-ve	Pseudomonas aeruginosa
2	+ve (Purple colour spehers in clusters)	Cocci	-ve	+ve	+ve	+ve	-ve	+ve	Staphylococcus aureus.
3	+ve (Purple rods)	Rod	-ve	-ve	Beta	-ve	-ve	-ve	Lactobacillus spp.

4	+ve (Purple elongate d rods occurrin g singly)	Rod	+ve	+ve	Beta	+ve	-ve	-ve	Bacillus spp.
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Microbial count and Percentage of Occurrence of Pure Isolates- The microbial count and the percentage of occurrence of pure isolates were determined. The table below shows that Bacillus spp. has the highest frequency while Lactobacillus spp. has the lowest frequency

Table 4: Microbial count and Percentage of Occurrence

Probable Organisms	Microbial count	Occurrence%
Bacillus spp.	1.9×10^6	90%
Pseudomonas aeruginosa	1.6×10^6	60%
Staphylococcus aureus	1.53×10^6	56%
Lactobacillus spp.	1.2×10^6	40%

5. DISCUSSION

The study was conducted to assess the microbial properties of soil receiving cassava effluents in cassava mills in two different mills in Ifite, Awka. Microorganisms in soils are being used as sensitive indicators of soil health and quality because of the relationships between microbial density, soil and ecosystem sustainability. (Ukaegbu-Obi et al., 2018) Cassava effluents are rich in nutrients especially sugars and thus, it is likely that microorganisms will thrive in such an environment where there is abundance of nutrients. (Ukaegbu-Obi et al. 2018) isolated Bacillus spp. and Pseudomonas spp.. in all the samples used in their research (with 100% occurrence in both the control and contaminated soil samples), while Staphylococcus spp. recorded 100% occurrence only in the control samples, with 60% occurrence in the contaminated soil samples and no records of Lactobacillus spp. Pseudomonas and Bacillus species were found to thrive well in the effluent contaminated soil. In the work of (Enerijiofi et al. (2017), bacteria isolates included Pseudomonas (15.48%), Bacillus (12.41%), Acetobacter and Rhizopus (10.88%), Corynebacterium (9.33%), Lactobacillus, Micrococcus, Staphylococcus (6.25%). The study documented a diverse range of microbial isolates with Pseudomonas (15.48%) being the most frequently occurred species. The preponderance of these isolates could be due to the biodegradable nature of cassava mill effluent. However, some of the microorganisms reported in this study had earlier been documented by previous researchers (Okechi et al., 2012) and (Omotioma et al., (2013) where they reported Proteusmirabilis, Proteus vulgaris, Staphylococcus saprophyticus, Bacillus, Klebsiella, Streptococcus species. These organisms, Bacillus, Klebsiella and Pseudomonas are considered as opportunistic pathogens that can cause wound infections leading to sepsis. The bacteria isolated from the studied areas in the research done by Igbinosa and Igiehon (2015) were Bacillus subtilis, Bacillus macerans, Pseudomonas aeruginosa, Klebsiella oxytoca and Escherichia coli. The total heterotrophic bacterial ranged from 1.3×10^8 - 3.61×10^8 CFU/g. In this current research, Bacillus spp. was the highest occurring microorganism with 90% occurrence in both the contaminated soil samples and the control sample while Pseudomonas spp. had a 60% occurrence in the contaminated soil samples and the control samples. This is similar to the research done by (Ukaegbu-Obi et al., 2018) where Bacillus and Pseudomonas species had the highest percentage. The bacteria colony count of the pure cultures ranged from 1.2×10^6 to 1.9×10^6 .

6. CONCLUSION

The results of the present study reveal that the cassava effluent has impacts on the microbial diversity of the receiving soil. This is indicated by the significant increase observed in the microbial density of the polluted soil and the simultaneous impact on the physicochemical parameters of the soil. The effluent from cassava plant when discharged on soil causes physicochemical and microbiological changes in the soil which calls for serious concern if the soil will be used for agricultural and other purposes. It is clear that most of these cassava mills are sited near residential areas. There is therefore the need for an introduction of regulations to control the disposal of effluent generated from cassava.

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