AEROBIC MICROORGANISMS ASSOCIATED WITH CASSAVA PEELS BIODEGRADATION IN MAKURDI METROPOLIS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: March 7, 2021 **Accepted:** July 18, 2021

Abstract: This research was aimed at investigating microorganisms associated with the biodegradation of cassava peels. Cassava peels collected from villages near Federal University of Agriculture Makurdi, were washed using tap water to free them of soil particles. 30 g of cassava peels were weighed and placed in polythene bags labeled A, B, and C for seven days for the biodegradation process at room temperature (28^{o}C). Physico-chemical parameters which include temperature and pH were determined progressively during the degradation period. Bacteria and fungi were isolated from degraded cassava peels by serial dilution and pour plate technique and identification was done biochemically using Bergey's Manual and Compendium of Soil Fungi. Isolates were screened for cellulase production on carboxyl methyl cellulose (CMC) agar. Results from this study showed that isolated bacteria include; *Bacillus* spp. (37.5%) the most predominant bacterial isolate, followed by *Streptococcus* spp., *Staphylococcus aureus*, and *Pseudomonas* spp. (4.2%)being the least predominant bacterial isolate. Fungal isolates includes *Aspergilus* spp. (25.0%) the most predominant isolate, moderately followed by *Rhizopus* spp., *Saccharomyces* spp., *Penicilium* spp., *Mucor* spp. and *Candida* spp. and *Trichoderma* spp. (3.6%) was the least predominant isolate from the degraded cassava peels. *Pseudomonas* spp., *Bacillus* spp., *Escherichia coli*, *Aspergilus* spp., *Peniciluim* spp., *Trichoderma* spp., and *Rhizopus* spp., were able to degrade Cellulose amongst other isolates from the degraded cassava peels. Statistically, there was no significant difference between the bacterial load and the fungal load during the degradation process ($P \leq 0.05$). It is therefore recommended from the result of this research that, susceptibility analysis should be carried out on the isolated organisms to determine their level of resistance to antibiotics, microbial analysis of other cassava products should be done for further studies. Cassava species should also be analyzed to determine whether the distribution of microbial species is dependent on the species of cassava used.

Keywords: Microorganisms, cassava peels, biodegradation, *Manihot esculentum*, physicochemical

Introduction

Cassava (*Manihot esculenta* Crantz) is very useful in food processing industries but its wastes had been having devastating effects on the environment. Cassava is among the main vitality providers for many African citizens (Ifeanyi and Ojiako, 2018). According to previous studies which affirmed that "in the 1980s, cassava was the fourth most important dietary source of calories produced within the tropics and it probably still holds that position due to its great importance in the diet in Africa" (Cock, 2011). In Nigeria, apart from being a unique source of energy supply, several other benefits of cassava include; delivering food security, job and employment, raw materials, among others. Garri, *fu-fu*, *lafun*, and tapioca are the traditional food recipes from cassava (Ezedinma *et al*., 2007). Also dried chips and pellets, starch, glucose syrup, ethanol, high quality cassava flour (HQCF), and glue for industrial use can be processed from cassava (FAO, 2017; Nnadozie, 2015).

However, dumping of cassava peels in the environment is a major source of environmental pollution. Different groups of microorganisms are associated with the biodegradation of cassava peels, some of which have positive roles like the production of cellulase enzymes. Cellulase enzymes produced from fungi and bacteria had been reported to have cellulose-degradation ability which has enhanced significant changes in the nutritional composition of cassava peel waste like increasing glucose, nitrogen (Michael and Obasola, 2015). Besides, cellulase enzymes help to reduce the breakdown time of cellulose in the environment (Schwarz, 2001). Besides, some microorganism play negative roles like some molds that produce endotoxins as they degrade on the cassava peels. Some of their activities can be of benefits to the environment because they can degrade the toxic substances that pose a danger to the environment into simple and harmless substances. This contributes a lot in the control of environmental pollution (Iranso *et al*., 2001). This present study is to determine the microorganism associated with the biodegradation of cassava peels.

Materials and Methods

Sample collection and preparation

Fresh cassava peels were collected from villages near Federal University of Agriculture Makurdi. The fresh cassava peels were washed using tap water to free them of soil particle. 30 g of cassava peels were weighed and placed in polythene bags labeled A, B, and C and this was allowed to stay for one week (seven days) for the biodegradation process to take place.

Determination of temperature and pH of the samples

The physico-chemical parameters (temperature and pH) of the samples were determined using a thermometer (MS Digital Thermometer) and a pH meter (ModelZapmeta.ng), respectively. Temperature was determined by dipping a thermometer in the samples and taking the readings as appropriate. pH was also obtained by dipping the pH meter in the degraded cassava peels and then taking the readings as appropriate.

Media preparation

All media Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Carboxyl Methyl Cellulose (CMC) Agar were prepared in accordance with the manufacturer's specification, homogenized and thereafter sterilized by autoclaving at 121^{0}C for 15 min at the pressure of 15 pounds per square inch in an autoclave. The media were then allowed to cool down to about 45^{0}C before use. Streptomycin (0.1%) was added to PDA to prevent bacterial contamination of the media.

Isolation, identification and characterization of bacteria

Bacteria were isolated from degraded cassava peels by serial dilution and pour plate technique on NA. The isolates were macroscopically examined for morphology and colony characteristics such as shape, surface, elevation, pigment, edge and opacity. The isolates were screened for cellulase production on carboxyl methylcellulose (CMC) agar (Shanker *et al*., 2011). The formation of clear zone around the colonies

indicated cellulose degradation. Microscopic examination was done by Gram staining and then viewed under oil immersion objective (x100 magnification) to see the Color, Shape. Biochemical tests such as Indole test, Catalase test, Citrate test, Urease test, TSI test and Coagulase test were carried out on the isolates. The isolates were then identified using Bergey's Manual of Systematic Bacteriology (Don *et al*., 2005). The identification of bacteria was based on morphological characteristics and biochemical test that was carried out on the isolates. Characterization was done according to the method proposed by Fawole and Oso (2004).

Isolation, identification and characterization of fungi

Fungi were isolated from degraded cassava peels by serial dilution and pour plate technique on PDA. The isolates were macroscopically examined for morphology and colony characteristics such as growth patterns, spore and mycelia colouration and distribution of spores. The isolates were screened for cellulase production on carboxyl methylcellulose according to the methods proposed by Shankar *et al.* (2011). Formation of clear zone around fungal spore indicated cellulose degradation. The microscopic examination was done by lactophenol cotton blue staining and viewed under x10 magnification, then x40 magnification to see the hyphae, spores and spore arrangement. Identification was based on Compendium of Soil Fungi (Domsch *et al*., 1980).

Result and Discussion

The biodegradation of cassava peels is a complex process which involves so many groups of Microorganisms, these include fungi and bacteria species which plays a very important role in the process. In this research the various groups of Microorganisms that were involved in the biodegradation of cassava peels were investigated and the findings are presented in the tables below. Table 1 shows the temperatures and the pH of the cassava peels during the biodegradation process from day one to day seven. Tables 2, 3 and 4 show the morphological characteristics of bacteria and fungi isolated in the study.

Table 1: pH and temperatures for sample A, B, and C on Day 1-7 respectively

Samples	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	Temp	pH	Temp	pH	Temp	pH	Temp	pH	Temp	pH	Temp	pH	Temp	pH
A	28.3^0C	5.50	27.2^0C	4.57	27.5^0C	3.57	27.4^0C	3.65	26.5^0C	3.42	26.4^0C	3.24	26.6^0C	2.69
B	28.1^0C	5.44	27.4^0C	4.12	26.9^0C	3.56	27.2^0C	3.57	26.2^0C	3.19	25.9^0C	2.94	26.6^0C	3.18
C	26.6^0C	5.91	27.9^0C	4.87	26.8^0C	4.05	26.6^0C	4.00	25.8^0C	3.29	25.9^0C	3.03	26.4^0C	3.21

Temp = Temperature

Table 2: Morphological characteristics of bacteria isolated on day one and day three

Samples	CFU/ml	Colour	Elevation	Margin	Shape	Suspected Organisms
A_{1a}	1.2×10^5	Ash and Milk	Convex	Undulate	Rod shaped	*Bacillus* spp.
A_{1b}	1.2×10^{11}	Milk	Convex	Entire	Cocci that occurred in chains	*Streptococcus* spp.
A_{3a}	3.1×10^4	Milk	Entire	Circular Punctiform and irregular	Rod shaped	*Proteus* spp.
A_{3b}	2.3×10^{10}	Milk	Erose, Entire Undulate	Irregular, Circular and Punctiform	Cocci that occurred in clusters	*Staphylococcus aureus*
B_{1a}	5.0×10^4	Milk	Convex	Filamentous	Rod shaped	*Bacillus* spp.
B_{1b}	1.4×10^{11}	Milk and yellow	Convex	Entire	Cocci that occurred in clusters	*Staphylococcus aureus*
B_{3a}	3.6×10^4	Milk	Curled and Entire	Punctiform Circular and Irregular	Rod shaped	*Escherichia coli*
B_{3b}	2.1×10^{10}	Milk, Yellow	Entire	Circular, Punctiform	Cocci that occurred in clusters	*Bacillus* spp.
C_{1a}	1.2×10^5	Milk	Convex	Entire	Rod shaped	*Escherichia coli*
C_{1b}	1.010^{11}	Milk	Pulvinate	Entire	Rod shaped	*Bacillus* spp.
C_{3a}	2.1×10^4	Milk	Undulate	Irregular and Punctiform	Cocci that occurred in chains	*Streptococcus* spp.
C_{3b}	1.0×10^{11}	Yellow, milk & Greenish	Undulate and Erose	Irregular, Circular	Rod shaped	*Escherichia coli.*

Table 3: Colony morphology of bacteria isolated on day six and day seven

Samples	CFU/ml	Colour	Elevation	Margin	Shape	Suspected organisms
A_{6a}	1.2×10^5	Milk	Convex	Entire	Rod shaped	*Pseudomonas* spp.
A_{6b}	7.7×10^{10}	Milk, Yellow	Pulvinate	Entire	Rod shaped	*Proteus* spp.
A_{7a}	1.0×10^5	MucoidPink, Milk	Raised, Pulvinate	Entire	Rod shaped	*Klebsiella*spp.
A_{7b}	7.7×10^{10}	Milk	Convex	Entire	Cocci that occurred in chains	*Bacillus*spp.
B_{6a}	7.0×10^4	Yellow, Greenish & White	Umbonate, Raised	Undulate, Curled, Erose	Rod shaped	*Bacillus*spp.
B_{6b}	1.1×10^{11}	Mucoid Pink, Milk	Umbonate	Undulate	Rod shaped	*Klebsiella*spp.
B_{7a}	3.8×10^4	White , Milk	Pulvinate, Convex	Entire	Rod shaped	*Bacillus*spp.
B_{7b}	7.210^{10}	Yellow, Milk	Convex	Entire	Cocci that occurred in clusters	*Staphylocccusaureus*
C_{6a}	6.8×10^4	White Milk and Yellow	Convex	Entire, Erose	Rod shaped	*Bacillus* spp
C_{6b}	8.0×10^{10}	Yellow and Milk.	Convex	Entire	Cocci that occurred in chains	*Streptococcus* spp.
C_{7a}	1.3×10^4	Milk	Convex	Undulate	Rod shaped	*Bacillus* spp
C_{7b}	6.8×10^{10}	Yellow , Milk	Convex	Entire, Erose	Cocci that occurred in chains	*Streptococcus* spp.

Table 4: Characteristics of the fungal isolates from lactophenol cotton blue stain

Samples	CFU/ml	Colour	Probable organism
A_{1a}	5.0×10^{3}	Black Surrounded by white moulds	*Aspergilus* spp.
A_{1b*}	6.0×10^{9}	Grey surrounded by White moulds	*Rhizopus* spp.
A_{1b**}	6.0×10^{9}	Flat, smooth, moist, glitering and cream in colour	*Saccharomyces* spp.
B_{1a*}	2.0×10^{4}	Green, light Pink surrounded by white mould	*Aspergilus* spp.,
B_{1a**}		Yellow surrounded by green moulds, milk	*Trichoderma* spp.
B_{1b}	1.3×10^{10}	Black, white and milk	*Rhizopus* spp.
C_{1a*}	5.0×10^{3}	Flat, smooth, moist, dull and cream in colour	*Saccharomyces* spp.
C_{1a**}	5.0×10^{3}	Blue-green, powdery and pale on reverse	*Aspergilus* spp.
C_{1b*}	6.0×10^{9}	Black surrounded by white moulds	*Aspergilus* spp.
C_{1b**}	6.0×10^{9}	Green surrounded by yellow mould.	*Penicilium* spp.
A_{3a*}	1.6×10^{4}	Green, white, light pink surrounded by white mould	*Aspergilus* spp.
A_{3a**}	1.6×10^{4}	Green surrounded by White mould	*Penicilium* spp
A_{3b}	2.3×10^{10}	White, green, dark pink	*Rhizopus* spp.
B_{3a}	6.0×10^{3}	Yellow, black, pinkish	*Mucor* spp.
B_{3b}	1.5×10^{10}	White, black, green	*Rhizopus* spp.
C_{3a}	7.0×10^{3}	Brown, ash, green	*Penicilium* spp.
C_{3b}	1.7×10^{10}	Green, white, light pink surrounded by white mould	*Aspergilus* spp.
A_{7a}	4.4×10^{4}	Light green	*Penicilium* spp.
A_{7b*}	9.0×10^{9}	Green surrounded by yellow, white mould	*Candida* spp.
A_{7b**}	9.0×10^{9}	Flat, smooth, moist, dull and cream in colour	*Saccharomyces* spp.
B_{7a*}	3.0×10^{3}	Pale grayish brown Surrounded by white mould	*Mucor* spp.
B_{7a**}	3.0×10^{3}	Green surrounded by white mould	*Rhizopus* spp.
B_{7b*}	2.0×10^{10}	Yellow, light brown	*Aspergilus* spp.
B_{7b**}	2.0×10^{10}	Pale grayish brown Surrounded by white mould	*Mucor* spp.
C_{7a*}	6.0×10^{3}	Pale Grayish Brown Surrounded by white mould	*Mucor* spp.
C_{7a**}	6.0×10^{3}	Yellow surrounded by white mould	*Rhizopus* spp.
C_{7b*}	1.5×10^{10}	Flat, smooth, moist, dull and cream in colour	*Saccharomyces* spp.
C_{7b**}	1.5×10^{10}	Green surrounded by white light brown mould	*Candida* spp.

CFU= Colony forming unit, ml= Milliliter

Table 5: Biochemical characteristics of bacterial isolates encountered in the study

Samples	Gram Reaction	Catalase Test	Urease Test	Indole Test	TSI Test	Coagulase Test	Citrate Test	Probable organism
A_{1a}	+	+	-	-	-	-	+	*Bacillus* spp.
A_{1b}	+	-	-	-	-	-	+	*Streptococcus* spp.
B_{1a}	+	+	-	-	-	-	+	*Bacillus* spp.
B_{1b}	+	+	+	-	-	+	+	*Staphylococcus* spp.
C_{1a}	-	+	-	+	+	-	-	*Escherichia coli*
C_{1b}	+	+	-	-	-	-	+	*Bacillus* spp.
A_{3a}	-	+	-	-	+	-	+	*Proteus* spp.
A_{3b}	+	+	+	-	-	+	+	*Staphylococcus* spp.
B_{3a}	-	+	-	+	+	-	-	*Escherichia coli*
B_{3b}	+	+	-	-	-	-	+	*Bacillus* spp.
C_{3a}	+	-	-	-	-	-	+	*Streptococcus* spp.
C_{3b}	-	+	-	+	+	-	-	*Escherichia coli.*
A_{6a}	-	+	-	-	+	-	+	*Pseudomonas* spp.
A_{6b}	-	+	+	-	+	-	+	*Proteus* spp.
B_{6a}	+	+	-	-	-	-	+	*Bacillus* spp.
B_{6b}	-	+	+	-	+	-	+	*Klebsiella* spp.
C_{6a}	+	+	-	-	-	-	+	*Bacilus* spp.
C_{6b}	+	-	-	-	-	-	+	*Streptococcus* spp
A_{7a}	-	+	+	-	+	-	+	*Klebsiella* spp.
A_{7b}	+	+	-	-	-	-	+	*Bacillus* spp
B_{7a}	+	+	-	-	-	-	+	*Bacillus* spp
B_{7b}	+	+	+	-	-	+	+	*Staphylococcus aureus*
C_{7a}	+	+	-	-	-	-	+	*Bacillus* spp
C_{7b}	+	-	-	-	-	-	+	*Streptococcus* spp

+= Positive, - = Negative

Table 5 reveals the biochemical characteristics of bacterial isolates. Tables 6 and 7 show the frequency of bacteria and fungi isolated in the study while Tables 8 and 9 show the reaction of bacteria and fungi respectively on Carboxylmethyl cellulose (CMC).

Table 6: Bacterial isolates from cassava waste peel

Bacteria	Frequency	Percentage (%)
***Bacillus* spp.**	9	37.5
***Streptococcus* spp.**	4	16.7
Staphylococcus aureus	3	12.5
Escherichia coli	3	12.5
***Proteus* spp.**	2	8.3
***Pseudomonas* spp.**	1	4.2
***Klebsiella* spp.**	2	8.3
Total	**24**	**100**

Table 7: Fungal Isolates from Cassava Waste Peel

Fungi	Frequency	Percentage (%)
Aspergilus spp.	7	25.0
Rhizopus spp.	6	21.4
Saccharomyces spp	4	14.3
Penicilium spp.	4	14.3
Trichoderma spp.	1	3.6
Mucor spp.	4	14.3
Candida spp.	2	7.1
Total	**28**	**100**

Table 8: Characteristics of bacterial isolates on CMC

Bacteria	Reaction
Bacillus spp.	+
Streptococcus spp.	-
Staphylococcus aureus	-
Escherichia coli	+
Proteus spp.	-
Pseudomonas spp.	+

CMC=Carboxylmethyl cellulose, += Positive, = Negative

Table 9: Characteristics of fungal isolates on CMC

Fungi	Reaction
Aspergilus spp.	+
Rhizopus spp.	+
Saccharomyces spp	-
Penicillium spp.	+
Trichoderma spp.	+
Mucor spp.	+
Candida spp.	-

CMC=Carboxylmethyl cellulose, += Positive, - = Negative

In this present study, fungi and bacteria associated with cassava peels biodegradation were assessed with *Bacillus* spp. constituting (37.5%) being the most predominant bacterial isolate. This was followed by *Streptococcus* spp. consisting (16.7%) and *Pseudomonas* spp. constituting (4.2%) being the least predominant bacterial isolate amongst the bacterial isolates. This is in agreement with the work of Elijah *et al. (*2014) who reported that *Bacillus* spp. is the dominant bacterial species in cassava peels waste. In reference to the works of Pandey *et al.* (2000) and Gupta *et al.* (2003), *Bacillus* spp. was the predominant bacteria and also known to produce the enzymes amylase which decomposes starchy compounds. *Aspergilus* spp. (25%) was the most predominant fungi isolate followed by *Rhizopus* spp. *(*21.4%*)* and *Trichoderma* spp. (3.6%) was the least predominant fungi isolate amongst the fungal isolates from degraded cassava peels.

A variety of microbial species play essential roles in the biodegradation of cassava peels. Adeleke *et al.* (2017) reported that *Bacillus* spp., *Lactobacillus* spp., *Aspergilus* spp., *Mucor* spp., *Penicilium* spp., *and Rhizopus* spp. were isolated from fermented cassava peels, the most occurring isolates throughout the fermentation process were *Bacillus* spp. and *Lactobacillus* spp. Ayansina *et al.* (2014) also reported that *Streptococcus* spp., *Lactobacillus* spp. and *Bacillus* spp. were the most predominant bacterial isolates. Studies have shown that many *Streptococcus* spp. and *Lactobacillus* spp. are normal floral of the cassava tuber (Arotupin, 2007).

The pH decreased from day one (1) to day seven (7), respectively. This indicated that there was decrease in alkalinity and increase in acidity of the degraded cassava peels; this can be attributed to the biodegradation activities of Microorganisms. This also was observed by Oyewole (1990) who revealed that pH of fermenting cassava tubers decreases with first 48 hours of fermentation which is due to organic acid produced by lactic acid bacteria. Temperature fluctuated throughout the degradation period probably because of the constant changes in climatic conditions throughout the period when biodegradation was carried out.

Pseudomonas spp., *Bacillus* spp., *Escherichia coli, Aspergilus* spp., *Penicilium* spp., *Trichoderma* spp. *Mucor* spp. and *Rhizopus* spp. were able to degrade and cause considerable changes in the nutrient composition of degraded cassava peels. This was possible due to their ability to utilize various organic substances present in the degraded cassava peels as sources of carbon and energy. *Bacillus* spp. has been reported to produce cellulase with activity on soluble and crystaline cellulose in the earlier work of Miranda *et al*. (2009). From the result presented, it could also be seen that *Pseudomonas spp.* degraded cellulose better than *Bacillus* spp. on Carboxylmethyl Cellulose (CMC) which agrees with the work of Sonia *et al.* (2013).

The abilities of *Trichoderma* spp. and *Aspergilus* spp. to produce hydrolytic enzymes such as cellulases has been earlier reported by Oksanen *et al.* (2000), Coral *et al.* (2002) and Onsori *et al.* (2005). The result of this work showed that *Aspergilus* spp. degraded cellulose better than *Trichoderma* spp. This disagrees with the work of Omojasola *et al*. (2008) who reported that *Trichoderma* spp. has higher performance than *Aspergilus* spp.

Conclusion

A wide range of Microorganisms are associated with the biodegradation of cassava peels. These microbial isolates may probably have originated from the soil, water and materials used during the cultivation and processing of cassava products. *Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Aspergilus* spp. *Rhizopus* spp. and *Saccharomyces* spp. are the most dominant organisms associated with cassava peels, biodegradation with *Bacillus* spp. being the most occurring bacterial isolate and *Aspergilus spp.* being the most occurring fungal isolate. It is therefore recommended from the result of this research that, susceptibility analysis should be carried out on the isolated organisms to determine their level of resistance to antibiotics, microbial analysis of other cassava products should be done for further studies. Cassava species should also be analyzed to determine whether the distribution of microbial species is dependent on the species of cassava used.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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