SOME MICROORGANISMS ASSOCIATED WITH SOILS EXPOSED TO CASSAVA (*MANNIHOT ESCULATUM*) PEELS

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Abstract

It is a common practice in many parts of the tropics to dump cassava peels in open fields. This work is aimed at isolating and characterizing major microorganisms associated with soils from such dump sites. Two separate soil samples exposed to cassava (*Manihot esculentum*) peels and another sample from a maize farm were collected from Iwo, South–West, Nigeria and analysed for pH, organic matter and microbial profile. Soils exposed to cassava peels had pH values of 9.63 and 9.45 while that from maize farm had pH value of 6.83. Organic matter contents for soil exposed to cassava peels were 2.74 and 3.92ug/g while that from maize farm was 2.0ug/g. Mean bacterial counts for soils exposed to cassava peels were $18x10^6$cfu/ml and $15x10^6$cfu/ml while that of maize farm was $2.5x10^6$cfu/ml; fungal counts in the same order were $8.8x10^4$cfu/ml, $7.9x10^4$cfu/ml and $1.6x10^4$cfu/ml. Frequencies of bacterial isolates from soils exposed to cassava peels were *Streptococcus* sp (17.7%), *Lactobacillus* sp (14.7%), *Bacillus* sp and *Corynebacterium* sp(11.8% each) and the least was *Agrobacterium* sp (5.9%). The findings revealed that cassava peels have the ability to serve as enrichment for the growth of some bacteria when present in the soil.

Keywords: cassava peels, microbial counts, *Streptococcus* sp, *Lactobacillus* sp

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Introduction

Nigeria is the largest world producer of cassava and cassava continues to be the most economic source of starch (Daramola and Osanyilusi, 2006). It is processed into such food as gari, fufu, lafun and "starch" (Ketiku *et al.,* 1978). Cassava is also used as a good roughage source for dairy, buffalo, goats and sheep by either direct feeding or as a carbohydrate source in the concentrate mixtures (Aderemi, 2010). Cassava waste generated during processing includes the peels which are often disposed on open dump sites near local processing factories. Domestic animals such as goats and sheep may feed on the peels which contains high levels of the toxic cyanide and may be of health hazard to many other animals.

Cassava peels may also pile up at dump sites thereby constituting nuisance in the environment and eventually leading to emission of offensive odors (Ubalua, 2007). The peels consist of the rough, brown outer part normally removed with some fleshy white or yellow part. The peels are therefore rich in starch and can be used in some industrial processes rather than the indiscriminate disposal. In view of its high contents of starch, microorganisms growing in the soil can utilize cassava peels as growth medium and in the process produce amylase as an extracellular enzyme to digest the starch (Gupta *et al.,* 2003).

Microorganisms that produce amylases are usually found in the immediate environments especially in places such as soils around mills, cassava farms and processing factories as well as flour markets (Fossi *et al.,* 2005).

In this study we investigated some major physico-chemical changes and microbial profile of soils from two cassava dump sites and a maize farm not exposed to cassava peels.

Materials and Methods

(i) Sample collection

Soil samples were collected randomly during the rainy season (between May & October, 2013) from two cassava dump sites in Iwo town (South-west, Nigeria) with sterile metal spoon at 0–30cm depth into clean polythene bags labeled A & B. Another sample (labeled C-control) was taken from the agricultural farm site of the Bowen University, Iwo, Osun State, Nigeria. The soil samples were kept in black polythene bags and sieve with 2.0mm mesh and kept for further analysis.

(ii) Soil physico–chemical parameters

a. **Soil pH –** This was determined by mixing 20.0g of soil sample with 20ml of distil water. The mixture was stirred with glass rod for 10mins and allowed to settle. The pH was then measured using the Jenway pH meter (model 3150).

b. **Soil organic matter –** This was determined as described by Jackson (1958) as modified by Ayansina and Oso (2008). Two grammes of oven-dried soil was weighed into a pre-weighed crucible in replicates. The crucibles were ignited over a Bunsen burner to red heat; and stirred occasionally with a steel rod for 15mins. The soils were allowed to cool in a desiccator before weighing.

(iii) Bacterial counts and identification

A modified method of the conventional serial dilution method was employed by mixing 20g of soil samples with sterile 180ml of sterile deionized water in 250ml conical flask. The mixture was shaken at regular intervals for about 20mins and allowed to settle. Serial dilution from the mixture was then done by pipetting 1ml into 9ml sterile deionized water in test-tube to the 6^{th} dilution (10^{-6}). One mililitre was transferred into sterile Petri dish. Warm sterile molten nutrient agar (oxoid) was then poured into the Petri dish, swirled gently and incubated at 37^0C for 24 hours. Two replicates of each were randomly made.

Biochemical tests employed in the identification and characterization of bacteria includes Gram's staining, catalase test, citrate test, MRVP test, motility test and sugar fermentation test.

(iv) Fungal counts and Identification

One milliliter each from the serial dilution was transferred into sterile Petri dish to which sterile molten potato dextrose agar (PDA – oxoid) was added and allowed to solidify. The plates were then incubated at 25^0C for 72 hours and observed daily for fungal out-growths. Sub–cultures were made to get pure cultures which were stored on agar slants and later for identification as described by Amadi and Adebola (2008) and Barnett and Barry (2010).

Results and Discussion

Values of some physico – chemical analysis carried out is presented in table 1. Soil samples from Cassava dump sites had higher pH values (9.63 & 9.45) compare to the soil samples from the university maize farm (6.83). The difference in the hydrogen ion concentrations can be as a

result of the varying soil composition; the university soil sample is an untreated and undisturbed soil which compensated for the near neutral pH. However the alkaline pH observed from the soils exposed to cassava peels must have resulted from the combinations of the activities enzymes from degrading microorganisms and other physic-chemical properties of the soil. Organic matter measurements also showed that soil samples from cassava dump sites had higher levels of organic matter (2.74 & 3.92ug/g) compared to soil sample from the maize farm (2.00ug/g). The decomposition of cassava peels must have resulted in the relatively high organic matter decomposition.

Major bacterial isolates from the soil samples from the university farm and cassava dump sites are presented tables 2, 3 and 4. It can be deduced from tables that introduction of cassava peels into the soils encouraged the growth of many species of bacteria i.e. serving as an enrichment medium for the growth of those microorganisms not found in the university farm soil. Major microorganisms isolated from the soils is further supported by results from soils exposed to cassava peels as shown in table 2. While the university farm soil sample had mean bacterial counts of 2.5 x 10^6 cfu/ml, soil samples from cassava dump sites had 18 x 10^6 and 15 x 10^6 cfu/ml. The same pattern was also observed for fungal counts. The active biodegradation of cassava peels in the dump sites must have accounted for the higher microbial counts compared to the soil from the university farm.

Frequency distribution of the major bacterial species isolated is shown in table 5. *Streptococcus* sp. was the most frequently isolated bacteria (17.7%). This was followed by the *Lactobacillus* sp. (14.7%). Studies have shown that many *Streptococcus* sp. and the *Lactobacillus* sp. are normal flora of the cassava tuber (Arotupin, 2007). Sohail *et al.* (2005) has shown that some *Lactobacillus* sp were producers of amylase as they also produce lactic acid during the fermentation of cassava. From this work *Bacillus* sp also had high frequency of isolation (11.8%). In reference to the works of Amund and Ogunsina (1987), Olafimihan and Akinyanju (1999), Pandey *et al.* (2000) and Gupta *et al.* (2003) *Bacillus* sp are also known to produce the enzymes amylase which decomposes starchy compounds. Other bacteria encountered are majorly normal flora of a typical tropical soil whose growth must have been encouraged by the presence of cassava peels.

Cassava processing into different foods is a common trade in many rural parts of Nigeria (Okafor, 1998). The peels regarded as wastes are usually discarded and allow to rotten away. Many researches are on to convert these cassava peels into many beneficial products such as animal feeds, compost, bio-fuel and ethanol. Das *et al.* (2000) reported that cassava peels can be utilized as a medium for mushroom cultivation or can be used to produce compost.

Table 1: pH and organic matter values of the soil samples

Sample	pH	O.M. (ug/g)
A	9.63	4.74
B	9.45	3.92
C	6.83	2.00

Table 2: Major bacterial isolates from farm the soil and cassava dumpsites

A	B	C
Staphylococcus sp *Agrobacterium* sp *Lactobacillus* sp *Streptococcus* sp *Pseudomonas* sp *Clostridium* sp *Bacillus* sp *Proteus* sp *Corynebacterium* sp Actinomycetes	*Staphylococcus* sp *Agrobacterium* sp *Lactobacillus* sp *Streptococcus* sp *Pseudomonas* sp *Bacillus* sp *Corynebacterium* sp *Actinomycetes*	*Agrobacterium* sp *Lactobacillus* sp *Bacillus* sp *Pseudomonas* sp

Table 3: Mean Microbial counts from soil samples analyzed

Soil Sample	Bacterial counts	Fungal counts
	$X10^6$ cfu/ml	$X10^4$cfu/ml
A	18	8.8
B	15	7.9
C	2.5	1.6

Table 4: Frequency of bacterial isolates from soils exposed to cassava peels

Bacterial species	Number	% Frequency (%)
Actinomycetes	12	8.8
Agrobacterium sp	8	5.9
Bacillus sp	16	11.8
Clostridium sp	10	7.4
Corynebacterium sp	15	11.0
Streptococcus sp	24	17.7
Lactobacillus sp	20	14.7
Proteus sp	11	8.1
Staphylococcus sp	9	6.6
Pseudomonas sp	11	8.1
Total	136	100

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