Microbiota Assay of Cocoa Pod Husk – Based Compost as Organic Fertilizer

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Abstract

Microorganisms play important roles in the recycling of agricultural wastes. Composting is the degradation of organic materials through the activities of diverse microorganisms. The compost generated by bioconversion of agro-residues offers several benefits such as enhanced soil fertility and soil health which can lead to increased agricultural productivity, improved soil biodiversity, reduced ecological risks and a healthier environment. This investigation examined the microbial community dynamics, loads and identification of microbiota at various stages of composting process. The compost produced from combinations of fresh and dry cocoa pod husk, Chromolaena odorata and cow-dung were assayed. Composts samples were randomly collected, isolation by standard serial dilution method and identification of microbes from compost materials were carried out at inception, 2, 4, 8 and 12 weeks after set-up of composting. Microorganisms isolated and characterized from the above composts include the species of bacteria viz., Pseudomonas spp., Staphylococcus spp., Streptococcus spp., Escherichia coli, Bacillus spp., Micrococcus spp., Proteus spp., Streptomyces spp., Enterobacter spp., Serratia spp., Salmonella spp. and Shigella dysenteriae and fungi viz., Trichoderma spp., Geotrichum spp., Chrysosporium spp., Aspergillus spp., Yeast spp., Absidia spp., Mucor spp., Rhizopus stolonifer, Penicillium spp., Fusarium spp., Phimben spp., and Microsporum spp. Of these isolates, Bacillus spp., Staphylococcus spp., E. coli and Streptococcus spp. are common to all stages of composting while Yeast, Mucor and Penicillium were the mycoflora common to all stages of the total microbial isolates. Composts supported high population level of bacteria (Bacillus 86.7%) while Yeast had 60% occurrence among fungi isolates. The microbial load of bacteria varied between 7x10⁶ cfu and 12x10⁶ cfu and 2x10³ cfu and 5x10³ cfu for fungi isolates. The associated isolates were highest (20 isolates) at 2 weeks of composting and the population decrease with maturity of the compost.

Keywords
Compost, Cocoa pod husks, Microbiota, Colony count, Fertility

Introduction
Composting is the biological conversion of solid organic waste into usable end products such as fertilizers substrates and biogas. Moreover, their high organic matter content and biological activity make composts effective in a variety of applications, including erosion control, re-vegetation, bio-filtration and bio-remediation (Anastasi, et
The active component involved in the biodegradation and conversion processes during composting is the resident microbial community, among which fungi and bacteria play very important roles (Anastasi et al., 2005). The biological process of composting comprises of the complete or partial degradation of a variety of chemical compounds by a consortium of microorganisms, the composition of which changes as composting progresses (Whitney and Lynch 1996). The microbial community during composting follows a predictable successional pattern resulting in the re-colonization of compost with metabolically active mesophilic populations that can be suppressive towards plant pathogens. Composting is a controlled aerobic process that degrades organic waste to stable material, with the resident microbial community mediating the biodegradation and conversion processes (Neher et al., 2013).

Microorganisms play very significant role in biodegradation of compost materials, bacteria and fungi species play significant roles in the decomposition and mineralization of agricultural organic wastes (Zhang et al., 2014). There are three distinct successional phases driving chemical and microbial changes through time, phases that are determined primarily by changes in temperature (Psychophilic) < 20°C: Mesophilic phase (moderate temperatures rising to 45°C), Thermophilic phase (high temperatures peaking 70°C), curing phase (cooling to ambient temperature) (Ryckeboer et al., 2003). Compost production is a critical component of organic waste handling, and compost applications to soil are increasingly important to crop production. The primary goals of composting have included the save handling of organic wastes and enhancement of soil’s fertility (Hubbe et al., 2010).

Various biological studies have been carried out to identify the major microbiological agents responsible for biodegradation (Taiwo and Oso, 2004). Macdonald et al., (1981) noted that the composting process was brought about by several organisms such as bacteria, fungi, actinomycetes and protozoa and may also involve invertebrates such as nematodes, pot worms, earthworms, mites and various other organisms. Singh (1987) however noted that the sole agents of decomposition of carbonaceous materials are the heterotrophic microorganisms some of which were cultured in this study.

Generally, successful composting depends on a number of factors that have both direct and indirect influence on the activities of the microorganisms. They include the type of raw materials being composted, its nutrient composition, moisture content, temperature, acidity or alkalinity and aeration. Composting is a fertilizing mixture of partially decomposed organic matter from plant and animal origin (Piet et al., 1990). The active component mediating the biodegradation and conversion processes during composting is the resident microbial community, among which fungi play a very important role. Therefore, optimization of compost quality is directly linked to the composition and succession of microbial communities in the composting process (Taiwo and Oso, 2004, Peters et al., 2000).

Cocoa pod husk (CPH), fresh and dry is generated in large quantities in cocoa farms in Nigeria. Nigeria produces annually about 8,000,000 tonnes of Cocoa Pod Husk (CPH), which are left to waste. By this it was estimated that 64000-94000 tonnes of nutrients like K, Ca and P and between 6000-9000 tonnes of N are lost annually (Egunjobi, 1975, Ajayi et al., 2007). Cocoa pod husk and its ash have not been adequately studied in plant nutrition. Ayeni et al., (2008a) reported cocoa pod ash contained plant nutrients as N, P, K Ca, Mg and micronutrients and is good for tomato production (Odedina et al., 2003, Macdonald et al., 1981).
Ayeni, 2010). These CPH either fresh or dry could be composted for use as organic fertilizer for cocoa or any other crop. The major difference between the fresh and dry cocoa pod husk is the water content.

Composts were made from fresh CPH, dry CPH and combination of both, while bacteria and fungi associated with composts of different sources were cultured. This study also carried out co-composting of (i) fresh CPH with Chromolaena odorata and cow dung (ii) dry CPH with Chromolaena odorata and cow dung (iii) dry CPH, fresh CPH with Chromolaena odorata and cow dung with a view to determine the microbiota status of each compost-type during composting and determine the nutrient contents and quality of the final compost.

Materials and Methods

Various composts were prepared using fresh, dry cocoa pod husk and combination of both as substrates. This research is a farmers’ participatory approach study carried out in Cross River State, Nigeria. Cocoa plantation at Effraya, Etung LGA, Cross River State was randomly selected. Participating farmers were involved in the choice of compost materials. Fresh and dry cocoa pods generated on cocoa plantation, Chromolaena odorata leaves were also harvested on adjacent plots and cow dung was sourced from the nearby Abattoir at Ikom, close to the experimental site. Necessary chopping and shredding was done as per requirement as it helps speed up decomposition and hasten the process of composting by increasing the surface area available for microbial action, and providing better aeration (Taiwo and Oso, 2004; Nielsen et al., 1997; Strom, 1985). The cocoa pod husks was weighed with Chromolaena odorata and cow dung in ratio 2:0.5:0.5 respectively and thoroughly mixed before packing into composting boxes. The experiment comprises of three compost combinations which were as follows: Fresh CPH + Chromolaena odorata + cowdung (2:0.5:0.5); Dry CPH + Chromolaena odorata + cowdung (2:0.5:0.5); Fresh CPH + Dry CPH + Chromolaena odorata + cowdung (1:1:0.5:0.5).

All the test composts were run in triplicate. The single and combination of substrates were prepared and monitored for composting outside for the period of 12 weeks at the end of cooling phase. Water was added until moisture content was adjusted between 40-60% (Buswell, 1984). Proper turning was done to get homogenous compost. As the composting progressed, the materials were regularly inspected using the traditional technique of touch and smell method. Moisture retention capacity of the compost was maintained and the temperature was noted successively till maturity.

Compost materials were randomly collected from the boxes, isolation of microorganisms was carried out at difference stages of composting viz., inception, 2, 4, 8 and 12 weeks after set-up and standard plate count (SPC) was performed according to Pelczar et al., (2003). Ten-fold serial dilutions were made up to $10^{-3}$ and $10^{-6}$. An amount of 1.0ml from the diluted samples were spread on Nutrient Agar (for bacteria) and Potato Dextrose Agar (for fungi) using a glass spreader. Petri plates were then incubated at ambient temperature for 24 h for bacteria and 4-5 days for fungi. The isolates were maintained on respective media slants. Prevalence of different groups of microorganisms was calculated in terms of appearance. These isolates were identified on the basis of conventional cultural and morphological characteristics of Barnett and Hunter, (1998); Barnett, 1960; and Brown (2005).

Results and Discussion

The research study shows a comprehensive
composition of microorganism in composts based on fresh and dry CPH substrates. The substrates utilized for composting are critical in composting and have impact on the type of active microbiota in the process. A total of 24 genera of microorganisms were isolated from composts at various stages of the process.

The bacteria isolates were *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia coli*, *Bacillus* spp., *Micrococcus* spp., *Proteus* spp., *Streptomyces* spp., *Enterobacter* spp., *Serratia* spp., *Salmonella* spp. and *Streptomyces* and the fungi include *Trichoderma* spp., *Geotrichum* spp., *Aspergillus* spp., *Yeast* spp., *Absidia* spp., *Mucor* spp., *Rhizopus stolonifer*, *Penicillium* spp., *Fusarium* spp., *Phimbens* spp. and *Microsporium* spp. The large majority (86.7%) occurrence was the genus *Bacillus*, followed by 73.3% occurrence of *Staphylococcus* while genus *Yeast* comprises of 60% of the total microbial isolates (Table 1). Substrates used in the study were composted singly and in combinations. It was observed that in comparison with the composts prepared from fresh single composites, combination of fresh CPH and dry CPH enhanced the diversity of saprophytic microorganisms that play an important role in the biodegradation process.

The microbial population of the composts varied with stages of composting, the isolate population was least by the 12th week (termination of the process) and highest of 20 genera of microbial isolates were recorded by at the 2nd week of composting activity. *Bacillus*, *Staphylococcus*, *E. coli*, and *Streptococcus* were the most prominent fungi across the sampling stages of composting; *Shigella dysenteriae* and *Chrysosporium* sp. were the least isolates present of the total microbial population (Table 1).

The study explains that when a microorganism is incubated in the presence of two or more materials, the materials will be degraded in the order of their ease of degradation.

Besides that, presence of two materials also increased the variety of microorganisms and that proper composting promotes the development of a number of saprophytic soil microorganisms.

Species of *Bacillus, Enterobacter, Flavobacterium, Pseudomonas, Streptomyces, Nocardia, Penicillium, Trichoderma and Micrococcus* have been reported by Anastasi *et al.*, (2005); Taiwo and Oso (2004) and Ryckeboer *et al.*, (2003) and that the cellulytic fungi such as *Geotrichum, Mucor, Microsporium, Fusarium, Rhizopus, Aspergillus, Trichoderma, Penicillium and Trichurus* accelerate composting for efficient recycling of crop wastes with high C: N ratio. Large and diversified microbial populations were found to be present during the composting process as well as in mature compost, and the appearance of some microorganisms stated above reflects the quality of maturing compost for good nutrients of plant use.

The Prevalence of the listed groups of microorganisms in composts based on multiple substrates is good indication of high quality compost.

Variations were recorded in the presence of microbial isolates at the sampling stages of composting with higher percent (57%) of bacteria recorded at composting initiation, 55% at 2 weeks of composting and 56% at 12 weeks. The fungi population was 56% and 60% at 4 and 8 weeks of composting activity respectively (Figure 1).
Table 1 Frequency of occurrence of microbial isolates in different stages of composting

| Microbial isolates          | Occurrence of microbial isolates in composting stages | Percent occurrence (%) |
|-----------------------------|--------------------------------------------------------|-------------------------|
|                             | Inception 2 weeks 4 weeks 8 weeks 12 weeks           |                         |
|                             | FPH DPH FDPH FPH DPH FDPH FPH DPH FDPH FPH DPH FDPH FPH DPH FDPH |                         |
| **Bacterial isolate on occurrences** |                                         |                         |
| Bacillus sp.                | + + + + + + + + + + + + + + + + + + + + + + + | 86.7                    |
| Staphylococcus sp.          | - + - + + + + + + + + + + + + + + + + + + + + | 73.3                    |
| Proteus sp.                 | - + - + + + + + + + + + + + + + + + + + + + + | 33.3                    |
| Streptococcus sp.           | - + + + + + + + + + + + + + + + + + + + + + + | 40.0                    |
| Micrococcus sp.             | - + - + + + + + + + + + + + + + + + + + + + + | 40.0                    |
| Serratia sp.                | - - - - - - - - - - - - - - - - - - - - - - | 13.3                    |
| Escherichia coli            | + - - + + + + + + + + + + + + + + + + + + + + | 5.3                     |
| Salmonella sp.              | - - + - - - - - - - - - - - - - - - - - - | 13.3                    |
| Enterobacter sp.            | + + - + + + + + + + + + + + + + + + + + + + + | 26.7                    |
| Shigella dysenteriae        | - - - - - - - - - - - - - - - - - - - - - - | 6.7                     |
| Streptomyces sp.            | - - - - + + + + + + + + + + + + + + + + + + | 13.3                    |
| Pseudomonas sp.             | - - - - + + + + + + + + + + + + + + + + + + | 13.3                    |
| **Fungal isolate occurrences** |                                         |                         |
| Trichoderma sp.             | - - - - - + + + + + + + + + + + + + + + + + | 26.7                    |
| Geotrichium sp.             | - - - + + - - - + + + + + + + + + + + + + + | 33.3                    |
| Chrysosporium sp            | - - - + + - - - + + + + + + + + + + + + + + | 6.7                     |
| Aspergillus sp.             | + + + + + + + + + + + + + + + + + + + + + + | 53.3                    |
| Yeast sp.                   | + - + + + + + + + + + + + + + + + + + + + + | 60.0                    |
| Absidia sp.                 | - - - - - - - - - - + + + + + + + + + + + + | 13.3                    |
| Mucor sp.                   | + - - - - - - - + + + + + + + + + + + + + + | 46.7                    |
| Rhizopus sp.                | + + - - - - - - - - - - + + + + + + + + + + | 20.0                    |
| Penicillium sp.             | - - + - - - - - - + + + + + + + + + + + + + | 33.3                    |
| Fusarium sp.                | - - - - - - - - - - - - + + + + + + + + + + | 13.3                    |
| Phimbens sp.                | - - - - - - - - + + + + + + + + + + + + + + | 20.0                    |
| Microsporium sp.            | - - - + + - - + + + + + + + + + + + + + + + | 33.3                    |
| **Number of isolates**       | 14 20 18 15 9  |                          |

*FPH: Fresh Pod Husk; DPH: Dry Pod Husk; FDPH: Fresh and Dry Pod Husk. Present (+); Absent (-).
Table 2 Inoculum load and identity of microbiota at the inception of composting

| Compost material | Microbiota associated with compost |
|------------------|----------------------------------|
| Fresh CPH        | Colony count (*cfu) | Bacterial isolates | Colony count (cfu) | Fungal isolates            |
|                  | 114.0 x 10^6 | Enterobacter sp, Bacillus sp, Escherichia coli | 60.0 x 10^3 | Mucor spp, Aspergillus spp, Yeast spp |
| Dry CPH          | 93.0 x 10^6 | Proteus sp, Staphylococcus sp, Bacillus sp, Micrococcus sp | 38.0 x 10^3 | Aspergillus sp, Rhizopus sp, Fusarium sp |
| Fresh + Dry CPH  | 68.0 x 10^6 | Streptococcus sp, Salmonella sp, Bacillus sp | 26.0 x 10^3 | Yeast spp, Penicillium sp, Aspergillus sp, Fusarium sp |

*Colony forming unit

Table 3 Inoculum load and identity of microbiota at 2 weeks after set up of compost

| Compost material | Microbiota associated with compost |
|------------------|----------------------------------|
| Fresh CPH        | Colony count (*cfu) | Bacterial isolates | Colony count (cfu) | Fungal isolates |
|                  | 128.0 x 10^6 | Pseudomonas sp, Staphylococcus sp, Streptococcus sp, Escherichia coli, Bacillus sp, Micrococcus, Enterobacter sp, Salmonella | 68.0 x 10^3 | Aspergillus sp, Geotrichum sp, Chrysosporium sp, Yeast sp |
| Dry CPH          | 65.0 x 10^6 | Proteus sp, Escherichia coli, Staphylococcus sp, Streptomyces sp | 37.0 x 10^4 | Trichoderma sp, Microsporium sp |
| Fresh + Dry CPH  | 130.0 x 10^6 | Staphylococcus sp, Enterobacter sp, Bacillus sp, Escherichia coli, Streptomyces, Serratia sp | 75.0 x 10^3 | Yeast sp, Mucor Phimbens, Penicillium sp |

*Colony forming unit

Table 4 Inoculum load and identity of microbiota at 4 weeks after set-up of compost

| Compost material | Microbiota associated with compost |
|------------------|----------------------------------|
| Fresh CPH        | Colony count (*cfu) | Bacterial isolates | Colony count (cfu) | Fungal isolates |
|                  | 66.0 x 10^6 | Pseudomonas sp, Shigella dysenteriae, Staphylococcus sp, Bacillus sp, Proteus sp | 42.0 x 10^3 | Trichoderma sp, Absidia sp, Yeast sp, Microsporium sp |
| Dry CPH          | 47.0 x 10^6 | Proteus sp, Bacillus sp, Staphylococcus sp, Serratia sp, Micrococcus sp | 43.0 x 10^3 | Rhizopus sp, Geotrichum sp, Penicillium sp, Mucor sp, Trichoderma sp, Microsporium sp, Aspergillus sp |
| Fresh + Dry CPH  | 147.0 x 10^6 | Staphylococcus sp, Escherichia coli, Proteus sp, Bacillus sp | 118.0 x 10^3 | Trichoderma sp, Geotrichum sp, Fusarium sp, Absidia sp, Yeast sp, Aspergillus sp, Mucor sp |

*Colony forming unit
**Table 5** Inoculum load and identity of microbiota at 8 weeks after set-up of compost

| Compost material | Colony count (*cfu) | Bacterial isolates | Colony count (cfu) | Fungal isolates |
|------------------|---------------------|--------------------|--------------------|-----------------|
| Fresh CPH        | 44.0 x 10^6 cfu     | *Streptomyces* sp, *Staphylococcus* sp, *Bacillus* sp, *Micrococcus* sp | 13.0 x 10^3 cfu | *Aspergillus* sp, *Geotrichum* sp, *Microsporium* sp, *Yeast* sp |
| Dry CPH          | 42.0 x 10^6 cfu     | *Escherichia coli*, *Bacillus* sp, *Staphylococcus* sp, *Streptococcus* sp | 22.0 x 10^6 cfu | *Rhizopus* sp, *Yeast* sp, *Fusarium* sp, *Mucor Plumbens*, *Penicillium* sp |
| Fresh + Dry CPH  | 41.0 x 10^6 cfu     | *Enterobacter* sp, *Bacillus* sp, *Escherichia coli*, *Staphylococcus* sp, *Streptomyces* sp | 16.0 x 10^4 cfu | *Mucor plumbens*, *Yeast cells*, *Geotrichum* sp, *Aspergillus* sp, *Microsporium* sp |

*Colony forming unit

**Table 6** Inoculum load and identity of microbiota at 12 weeks after set-up of compost

| Compost material | Colony count (*cfu) | Bacterial isolates | Colony count (cfu) | Fungal isolates |
|------------------|---------------------|--------------------|--------------------|-----------------|
| Fresh CPH        | 12.0 x 10^6 cfu     | *Staphylococcus* sp, *Micrococcus* sp, *Bacillus* sp | 5.0 x 10^3 cfu | *Yeast* sp, *Penicillium* sp |
| Dry CPH          | 9.0 x 10^6 cfu      | *Bacillus* sp, *Streptomyces* sp | 5.0 x 10^3 cfu | *Mucor* sp |
| Fresh + Dry CPH  | 7.0 x 10^6 cfu      | *Escherichia coli*, *Micrococcus* sp | 2.0 x 10^3 cfu | *Geotrichum* sp |

*Colony forming unit

**Fig.1** Population load of microbiota associated with compost
The microbial load of compost substrates varied at different stages of composting and the population of the isolates also differ based on the substrates and duration of compost.

The report of Nakasaki and Ohtaki (2002) indicates that the substrates will be degraded in the order of their ease of degradation when a microorganism is incubated in the presence of two or more substrates. The presence of two substrates also increased the variety of microbes and the higher percentage occurrence of fungi isolates in this study is corroborated by the earlier findings of Rabia et al., (2007) that fungal species were found to be numerous during both mesophilic and thermophilic phases of composting and that the appearance of some microorganisms reflects the quality of maturing compost (Strom, 1985).

The population of both bacteria and fungi were highest in the fresh CPH based compost at the inception of composting. Fresh and dry CPH based compost had the least population of bacteria and fungi. These were the initial inoculums that initiated the decomposition of the materials and they were mostly heterotrophic bacteria. Bacillus spp. of bacteria and Aspergillus spp. of fungi occurred in all the compost types at this stage. At inception of composting, bacteria load of 114x10^6 cfu/ml was recorded in fresh CPH while fungi colony was 60x10^3 cfu/ml in the same substrate. Bacillus spp. and Aspergillus spp. were recorded in all substrate type, but presence of Streptococcus, Salmonella, Penicillium and Fusarium were recorded in combination of substrates (Table 2). Similar observations were recorded on Seritia sp., Plumbens and Penicillium spp. (Table 3), E. coli and Fusarium spp. (Table 4), Enterobacter (Table 5), E. coli and Geotrichum spp. (Table 6). At two weeks after set-up of compost, the population of bacteria and fungi in the fresh and dry CPH based compost were highest. The bacterial and fungal isolates at the inception of composting also occurred two weeks after set-up, however Serratia spp and Pseudomonas spp were bacteria that surfaced in 2 weeks after while fungi like Trichoderma spp, Geotrichum spp and Chrysosporium spp were isolated newly at 2 weeks after set-up (Table 3).

Biodiversity of microbial populations were recorded at different stages of composting process and also in the mature compost. It has been suggested that the appearance of some microorganisms reflects the quality of maturing compost (Strom, 1985).

Shigella dysenteriae was isolated newly and only at 4 weeks after set up of compost in fresh CPH based compost. Pseudomonas spp of bacteria occurred only in fresh CPH based compost at 2 and 4 weeks after compost set-up. Population of bacteria and fungi in the fresh and dry CPH based compost were highest at 4 weeks after the setting up of the compost (Table 4).

At 8 weeks after compost set-up, no new bacterial isolate was found in all the compost types (Table 6). The populations of bacteria at 8 and 12 weeks after compost set-up were highest in fresh CPH based compost compared to other types of compost (Tables 5&6).

The populations of bacteria and fungi at 8 and 12 weeks were lower than what obtained at 2 and 4 weeks after compost set-up. This might be due to the fact that at 2 and 4 weeks, decomposition of the composting materials was at the peak and require more bacteria and fungi at this stage for decomposition than at 12 weeks when the compost was getting matured. Association of Aspergillus,
Trichoderma, Mucor, Penicillium, Alternaria, Cladosporium, Monilia, Helminthosporium, Coccidioides, Scedosporium with different composts were reported by Rabia et al., (2007). Jeanine et al., (2002) and Gbolagade (2006) also reported a wide range of bacteria viz., Pseudomonas, Bacillus spp., Serratia, E. coli, Micrococcus roseus, Citrobacter freundii, Clostridium perfringens, Klebsiella, Salmonella spp. and Enterobacter were been associated with compost, and most of these isolates were also identified in this study.

Gbolagade, (2006); Anastasi et al., (2005); Charest et al., (2004); Taiwo and Oso (2004) among others have reported the association of species of Bacillus, Enterobacter, Flavobalstitionum, Pseudomonas, Streptomycyes, Nocardia, Rhodococcus, Penicillium, Trichoderma and Gliocladium composting process. This corroborates the findings in this study as most of these microbial isolates were equally cultured and identified in this investigation.

In conclusion, the populations of bacteria and fungi at 8 and 12 weeks were lower than what obtained at 2 and 4 weeks after compost set-up. This might be due to the fact that at 2 and 4 weeks, decomposition of the composting materials was at the peak and require more bacteria and fungi at this stage for decomposition than at 12 weeks when the compost was getting matured. The combination of fresh and dry cocoa pod husks enhanced not only the number but also the diversity of saprophytic microorganisms that play important role in the biodegradation of the materials at 2 and 4 weeks after compost set-up. Conventional techniques were used to identify the fungal cultures however molecular techniques can be adopted to have a better understanding of active compost fungi. Along with the systematic characterization of fungal communities in compost, a functional analysis is needed to highlight potentials and applications as large unexploited diversity of microorganisms awaits discovery.

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