Isolation and Identification of Microorganisms Associated with Domestic Food Wastes from a Dumpsite in Akure, Ondo State, Nigeria

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Accepted on June 25, 2012

Abstract
This study aimed at the isolation and identification of different microorganisms that were associated with the domestic food wastes at the Ondo State Waste Management Board Dumpsite in Akure. The microorganisms were isolated using standard microbiological and biochemical methods. Microbial population of each sample was determined using the pour plate method with nutrient agar (NA) and potato dextrose agar (PDA). The NA and PDA plates were kept at 37°C for 24 hours (bacteria) and 28°C for 7 days (fungi). Bacterial colonies and fungal spore forming units obtained were counted and studied for cultural, microscopic and biochemical traits. The bacterial population of matured compost was high (5.3×10^6 Cfu/g) relative to those of newly mixed wastes (7.9×10^5 Cfu/g) and dried matured compost (5×10^4 Cfu/g). The fungal load of the newly mixed wastes was the highest (4×10^4 Sfu/g) compared with others that were 2×10^4 Sfu/g and 3×10^4 Sfu/g for dried and mature composts, respectively. Nine bacteria were identified as Azotobacter species, Bacillus megaterium, B. sphaericus, Geobacillus stearothermophilus, Kurthia spp., Macromonas mobilis, Lactobacillus delbrueckii, L. jensenii and Listeria monocytogenes. The six fungi isolated were Amblyosporium botrytis, Geotrichum albidum, Gloeosporium nervisequum, Sepedonium ampullosporum, Streptothrix atra and Variscosporium elodeae. The different microorganisms found to inhabit the domestic food wastes in this study are similar to the microorganisms (Bacillus, Azotobacter and fungi) that have been found associated with the transformation of compost to humus and could be useful in the bioconversion of domestic food wastes into soil amendments and composts, for agricultural purposes.

Keywords: *Bacteria, fungi, microbial population, domestic food wastes.*

Introduction
Food waste is any food substance, raw or cooked, which is intended or required to be discarded (Defra, 2009). Food waste makes the largest component of discarded wastes resulting from food preparation, leftovers, spoilt foods, poor preparation of foods, refrigerator or freeze accidents that occurred on foods due to power failure, over purchasing and expiry dates of foods (Miller, 2004; Knipe, 2005). Domestic food waste (DFW) is generated in homes, restaurants and markets. It could also be from residences and commercial establishments such as grocery stores, produce stands, institutional cafeterias, kitchens and industrial sources (EPA, 2006). The report of the Ondo State Waste Disposal Management Board in Akure metropolis stated that 1.2-1.5 tonnes (per day) of various food wastes are generated domestically in the environment and are deposited at the dumpsite in the town. These wastes are similar to some agricultural wastes.

High amounts of lignocellulose's agricultural residues including vegetable materials, fruits and foodstuffs generated commonly in homes, offices, markets, restaurants, agricultural and food processing industries are indiscriminately dumped at dumpsites and in landfills (Miller, 2004).

Microorganisms are found everywhere in the world and their association depends on the growth conditions of an environment. Microorganisms are critical to nutrient recycling in ecosystems as they act as decomposers. They are responsible for building fertile soil for plants to grow in; thus, microbes stick to the roots of plants and decompose dead organic matter into food for the plant to absorb (Wikipedia, 2010). Some microbes fix nitrogen, as a vital part of the nitrogen cycle (Christner *et al.*, 2008). Wastes are heavily colonized with various kinds of microorganisms for co-activities since waste is a biodegradable matter. Microorganisms act on organic material such as plant and animal matters and other substances originating from living organisms or similar artificial materials that are used by microorganisms (Todar, 2008).

Domestic food waste (DFW) is an organic matter which harbours an array of microorganisms. These microorganisms are usually commensals that are dependent upon two or more organisms (acting sequentially or simultaneously) for co-activities to decompose the different available nutrients. In order to identify the microorganisms resident in the DFW and compare them with those in similar wastes possible use in the transformation of wastes into useful material (such as compost) particularly for plant's use; this work was designed as a preliminary research to isolate and identify the microorganisms that are associated with DFW dumped at Ondo State Waste Disposal Management Board in Akure.

Materials and Methods

Collection of Samples

The domestic food wastes were obtained from the General Dumpsite of the Ondo State Waste Management Board situated at Akure North Local Government Area, along Oda Road, Akure (7.1° N, 5.3° E). It is a major dumpsite for domestically generated wastes. Newly mixed wastes, fresh mature compost and dried matured composts were collected aseptically at four different locations per heap culture in the weed row of the food wastes in the dumpsite. The samples were labelled thus: A, B and C respectively.

Isolation and Identification of Bacterial and Fungal Isolates

Ten grams of each sample collected were weighed into 90ml of sterile distilled water and shaken vigorously. One millilitre was taken from respective sample into 9ml of sterile water to make appropriate serial dilution of the waste samples. An aliquot (0.1ml) of the each serial dilution was pour plated with a molten nutrient agar (NA) and potato dextrose agar (PDA). The bacteria inoculated on NA plates were incubated at 37°C for 18-24 hours and the PDA pour plated with fungi were kept at 25-27°C for 72-120 hours. Then, streak plate technique was used to purify the bacterial isolates on NA while cork borer was used to transfer the fungal mycelia onto PDA to obtain pure culture of the fungi (Gabriel and Akharaiyi, 2007). The bacteria were examined for pigmentation, colony shape, edge, surface texture, elevation and consistency. The bacteria were morphologically studied for Gram reaction, cell shape, spore formation and position in the cell. The biochemical features examined on the bacteria were production of catalase, coagulase and urease with citrate utilization and sugar fermentation (Holt *et al.*, 1994).

The fungi were prepared for microscopic examination by placing 2 drops of lactophenol-in-cotton blue stain on a clean grease-free microscopic slide. A small piece of mycelium was removed from the medium with sterile inoculating needle. The mycelium was teased (picked) out with the needle, transferred onto the slide containing the stain and covered with a clean cover slip (Fawole and Oso, 2001). Identification of moulds was done by comparing the morphological characteristics observed microscopically as described by Onions *et al.* (1995).

Results and Discussion

The bacterial population of mature compost was high (5.3×10^6 Cfu/g) relative to those of newly mixed wastes (7.9×10^5 Cfu/g) and dried matured compost (5×10^4 Cfu/g). The highest bacterial population obtained in the fresh mature compost indicates that the microbes adjusted, utilized the available various kinds of nutrients present in the wastes for their metabolic activities and replicated (Table 1).

Table 1: Bacterial and fungal populations of domestic food wastes samples

Sample	←---------Total Population-----------→	
	Bacteria	**Fungi**
	($\times10^4$ Cfu/g)	($\times10^4$ Sfu/g)
Newly mixed wastes	79	4
Fresh mature compost	532	3
Dried matured compost	5	2

This is possibly due to the fact that the compost was fresh containing high amount of moisture and rich in nutrients to support the growth of the microorganisms. The dried matured compost had the lowest microbial population for both bacteria and fungi count, and this was an indication that the nutrients in the sample were not readily available for utilization due to lack of water in the compost had diminished as a result of the microorganisms present that has utilized the nutrients in the sample.

The fungal loads of the newly mixed wastes was the highest (4×10^4 Sfu/g) compared with others that were 2×10^4 Sfu/g (dried matured compost) and 3×10^4 Sfu/g (mature compost). The fungal populations of the mature and dried matured composts that were lower than the newly mixed wastes is probably be as a result of reduction in water content of the wastes for the fungi to thrive. Fungi have a lower water activity relative to that of bacteria (Omoya and Akharaiyi, 2008).

A total of nine bacterial and six fungal species were isolated and identified from the domestic food wastes (Tables 2 and 3).

Table 2: Cultural, morphological and biochemical characteristics of bacteria isolated from domestic food wastes in Akure.

Sample	S/No. of Isolate	←Cultural characteristics→						←Morphological characteristics→			←Biochemical characteristics→					←Sugar fermentation→							Tentative identity of bacteria
		Pigmentation (colour)	Shape	Edge	Elevation	Consistency	Surface texture	Gram reaction	Cell shape	Spore formation/ position in the cell	Motility	Catalase	Coagulase	Citrate utilization	Urease production	Glucose	Lactose	Sucrose	Mannitol	Salicin	Arabinose	Inositol	
A	A*	Yellowish white	Serrated	Undulate	Flat	Glistering	Dry	+	Rod	-	-	+	-	+	+	A	-	S	-	A	-	-	*Azotobacter* species
	A1	Cream	Circular	Entire	Flat	Butyrous	Dry	+	Rod	-	+	+	-	+	+	A	-	A	-	A	-	-	*Bacillus megaterium*
	A2	Yellowish white	Spine-round	Pointed	Raised	Mucoid	Wet	+	Rod	Terminal	+	+	-	+	+	S	-	A	-	A	-	-	*Geobacillus stearothermophilus*
	A^1_4	Creamy yellow	Filamentous	Entire	Convex	Butyrous	Wet	+	Rod	-	+	+	-	+	-	A	-	A	-	S	-	-	*Macromonas mobilis*
B	B1	Yellow	Round	Entire	Raised	Butyrous	Wet	+	Rod	-	+	+	-	+	+	-	-	-	-	A	-	-	*Kurthia* species
	B2	Yellowish white	Spear-mouthed (Lansolate)	Pointed	Raised	Butyrous	Dry	+	Rod	-	+	+	-	+	+	A	-	S	-	A	-	-	*Bacillus sphaericus*
C	C11	Creamy yellow	Irregular	Smooth	Flat	Swampy	Wet	+	Rod	-	-	+	-	+	+	S	-	S	-	-	-	A	*Lactobacillus delbrueckii*
	C12	Creamy yellow	Round	Entire	Flat	Butyrous	Dry	+	Rod	-	+	+	-	-	+	A	-	A	-	A	-	-	*Lactobacillus jensenii*
	C2	White	Punctiform (too tiny)	Rhizoid/ Spindle	Raised	Fluorescence	Wet	+	Rod	-	+	+	-	+	-	A	-	-	-	-	-	-	*Listeria monocytogenes*

Legend: +: Present/positive; -: Absent; S: Slight acid production; A: Acid production.

Table 3: Characteristics of fungi isolated from domestic food wastes in Akure.

Sample	S/No.	Cultural characteristics: Surface	Cultural characteristics: Reverse face	Microscopic appearance	Probable fungal identity
A	F1	White and fluffy	Light black	Conidiophores were indefinite not differing much from branched of the mycelium, simple or branched. Conidia were single or in loose clusters, hyaline and I-celled tuberculate.	*Sepedonium ampullosporum*
	F2	Creamy yellow and fluffy	Green	Mycelium was dark. Conidiophores were erect, tall, branched, branches spirally coiled (appearing wavy). Conidia were single, apical or lateral with short peg-like structures and I-celled.	*Streptothrix atra*
B	F3	Light brown and fluffy	Light brown	No sharp distinction between conidiophores and conidia. Conidiophores were simple or sparingly branched near the apex bearing conidia apically. Conidium consisted of a main elongated axis with 2 or 3 laterals. Each lateral was septate and branched.	*Variscosporium elodeae*
	F4	White and fluffy	Chocolate brown	Mycelium was white and septate but no conidiophores. Conidia were hyaline, I-celled, short cylindrical with truncated ends, formed by segmentation of hyphae.	*Geotrichum albidum*
C	F5	Green, carpet-like layer with white edge	Dark brown	Mycelium appeared pale to yellow–orange; conidiophores were erect, septate have unbranched portion bearing a number of irregular branches near the apex. Conidia were in chains formed by segmentation; the conidia were I-celled, hyaline and barrel-shaped.	*Amblyosporium botrytis*
	F6	White and fluffy	Light yellow	Disc-shaped or cushion–shaped. Conidiophores were simple but variable in length. Conidia were hyaline, I-celled, ovoid to oblong	*Gloeosporium nervisequum*

Legend:

A: Newly mixed wastes

B: Fresh mature compost

C: Dried matured compost

The bacterial growth was prominent on the nutrient agar plates between 18 and 24 hours. Most of the colonies were cream in colour and butyrous, while some showed variations in consistency, edge, elevation, shape etc. All the bacteria isolated were Gram positive bacilli, catalase positive, negative arabinose fermentors and non-spore formers except one, with a terminal spore. The bacteria isolated were *Azotobacter* spp., *Bacillus megaterium*, *Geobacillus stearothermophilus, Macromonas mobilis, Kurthia* spp., *Bacillus sphaericus, Lactobacillus delbrueckii, Lactobacillus jensenii* and *Listeria monocytogenes* (Table 2). The fungal grew with a fluffy surface. They possessed different colours and shapes for the reverse background. The fungi isolated were *Amblyosporium botrytis, Geotrichum albidum, Gloeosporium nervisequum, Sepedonium ampullosporum, Streptothrix atra* and *Varicosporium elodeae* (Table 3).

The different genera of microorganisms found to inhabit the domestic food wastes in this study were similar to the microorganisms (*Bacillus*, *Azotobacter* and fungi) associated with the transformation of compost to humus (Diver, 1999). Some of these microorganisms (Actinomycetes, *Azotobacter* and *Nitrosomonas* groups) were reported by Diver (1999) for the transformation of compost to humus. They were able to convert simple compounds into complex humic substances. He said that these microorganisms aid in the decomposition and mineralization of organic matter present in the food wastes thus influencing plant nutrition, in order to improve soil fertility (Diver, 2002).

The origins of the microbes identified in this work are wastes from our homes, markets, soils, farm produces and industries. Certain strains of *Lactobacillus* species, one of the microbial isolates, are found in dairy and meat products, sewage, beer, fruit juices, pickled vegetables and waste water effluents. Some *Lactobacillus* species are parasites inhabiting human mouth and vagina (Holt *et al.*, 1994). It was also reported by Holt *et al.* (1994) that *Azotobacter* is a mesophilic inhabitant of soil and water. The pathway of the bacilli isolated in this research work, could be traced to the farm wastes derived from yam, plantain, maize and vegetables which provided a rich substrate for the organism to thrive. *Bacillus* spores are common in agricultural soils and may be transferred from the soil environment to the farm produce. *Bacillus* species particularly those that originated from soils are known to participate in denitrification (Todar, 2008). The occurrence of *Geobacillus stearothermophilus* in compost has been documented. It also occurs in soil, hot springs, desert sand, ocean sediments and food (Todar, 2008). *Listeria monocytogenes* is associated with soil, silage, raw and pasteurized fluid milks, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, smoked fish, raw and cooked poultry (Dykes and Dworaczek, 2002). These microorganisms aided in the decomposition and mineralization of organic matters present in the food wastes by making use of the nutrients available in the compost, during which they release nutrients which are taken up by plant roots (Inckel *et al.*, 1999).

Conclusion

Microorganisms proliferate in waste due to the available nutrient constituents that support their growth (Giller and Cadisch, 1997). Microorganisms respond to the presence of organic materials by growing rapidly and using the easily available contents of the organic material. Thus, biological decomposition began as vegetation falls to the ground;

it slowly decays, providing minerals and nutrients needed for plants, animals and microorganisms (Mansour and Shaaban, 2007). Though, all constituents present in waste do not give equal percentage growth support to all microorganisms present in their locations.

Based on these points and the reported results of some scientists that some microbes found in wastes can convert the wastes to composts, the microbes isolated in this study could be useful in the decomposition of the DFW to compost for plants use. This decomposition will prevent accumulation of wastes and environmental pollution.

Acknowledgements
We acknowledge the Federal University of Technology, Akure, Nigeria for the provision of equipment and materials used for this research. The efforts of Mr. F. C. Akharaiyi towards the success of the laboratory analyses are greatly appreciated.

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