

**Isolation and Identification of Bacteria Associated with the Compost  
Generated from Cocoa Pod Husk and Cow Dung**

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**Abstract**

Generation of compost from organic materials has been in practice for numerous decades. This study was conducted to isolate and identify bacteria responsible for the composting of Cocoa pod husks (CPH) and Cow dung (CD). Ten kilogram of fresh Cocoa pod husks and 10.0 kg of Cow dung were composted for twenty days with a view to generating compost from the mixture. One gram of the compost was weighed and suspended into three test tubes containing 9.0 ml sterile distilled water. The mixture was vigorously shaken to give room for the formation of supernatant. Subsequently, 1.0 ml of the supernatant was introduced into test tubes containing Mineral Salt Medium (MSM). The mixture in the test tubes was then shaken vigorously and allowed to settle. The test tubes were subsequently incubated at 37.0 °C for four days and observed for bacterial growth indicated by broth medium turbidity. Bacterial growth observed after incubation was sub-cultured into three test tubes containing Trypticase Soy broth (TSB) and incubated at 37.0 °C for 48 hours. Further subculturing was carried out using three Trypticase Soy Agar (TSA) plates at 37.0 °C for 48 hours and colonies (C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>7</sub> and C<sub>8</sub>) that developed were observed and recorded. Bacterial colonies were subsequently picked and transferred to other glass slides to form smears. Gram staining procedures and biochemical tests were subsequently conducted. The results of the biochemical tests conducted on C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>7</sub> and C<sub>8</sub> indicated *Alcaligenes faecalis*, *Streptococcus* sp, *Serratiamarcescens*, *Pseudomonas* sp, *Flavobacterium* sp, *Escherichia coli*, *Bacillus stearothermophilus* and *Bacillus subtilis* as the isolated bacteria. However, molecular identification of the bacterial isolates is recommended with a view to further confirming their identity.

**Keywords: Composting, Cocoa pod husk, Cow dung, Bacterial isolates, Biochemical tests**

**1. Introduction**

Apart from microbial break down of organic materials principally for microbial metabolic activities, it has been reported that microorganisms that are

indigenous in a typical compost heap help in the restoration of the natural cycling of nutrients in the soil leading to the production of soil organic matter\_(Adegunloye and Olorunnusi, 2016). Compost generated

from the composting of organic materials has been documented to be rich in nutrients that can adequately enhance crop growth (Jin-Hyung *et al.*, 1996), very cheap as it is principally made of waste materials, renewable and can conveniently substitute the use of inorganic fertilizers that are somehow out of reach for local farmers (TNAU, 2009).

According to Oladokun (1995), *Theobroma cacao* is known as an important tropical rain forest species grown for its oil rich seeds that produce cocoa and cocoa butter. The author reported further that a typical cocoa pod houses about 42% beans, 2% mucilage and 56% pod husk. Raymond and Ray (1992), gave a breakdown of beef cattle waste composition as 2.8 % N, 1.0 – 2.0 % P, 1.0 – 3.0 % K, 1.5 % Mg, 1.0 – 3.0 % Na and total soluble salts 6.0 – 15.0 %. The application of cow dung in the correct proportion has the tendency of improving soil pH, organic matter, available phosphate and exchangeable cations of the soil. Its application to soil also increases all agronomic variables of crops (Jin-Hyung *et al.*, 1996).

Composting is the biological transformation of organic wastes, under the influence of controlled conditions, into a hygienic, humus-rich, comparatively biostable product that improves land and ultimately improves plant growth (Mathur, 1991). It is an arduous process involving degradation of organic waste aerobically through the metabolic activities of microorganisms; bacteria, fungi and actinomycetes (Hassen *et al.*, 2001; Ryckeboer *et al.*, 2003; Anastasi

*et al.*, 2005; Bhatia *et al.*, 2013; Pan and Sen, 2013). However, bacteria are responsible for most of the decomposition and heat generation that occur in a typical compost pile (Trautmann and Olynciw, 1996) and this is due to the ability of bacteria to employ a wide range of enzymes to break down organic materials chemically (Sonia *et al.*, 2002; Kutu *et al.*, 2019). Efficient composting requires optimum conditions that ensure optimum growth of the microorganisms aiding the process (Adegunloye *et al.*, 2007).

Composting has ability to combine the effect of pathogen reduction with stabilization and conversion of organic wastes into product that can be simply touched and applied to improve soil fertility (Martin and Gershuny, 1992; Zhu, 2006). Composting is one of the needed sanitization processes to lessen animal wastes' microbial load. The efficiency of composting in ensuring pathogens' inactivation greatly depends on allocated time and generated temperature. Ineffective composting process harbours tons of pathogenic bacteria which may be invariably passed on to the end users (Adegoke *et al.*, 2016). This study was conducted with the aim to isolate and identify the bacteria involved in the composting of cocoa pod husks and cow dung.

## **2. Materials and Methods**

### **2.1. Collection of Fresh Cocoa Pod Husks and Cow Dung**

Thirty kilogram (30.0 kg) of fresh cocoa pod husks (CPH) was collected from cocoa pod

husk heaps inside one of the cocoa plantations located at Irasa community, Ado Ekiti, Ekiti State. The cocoa plantation where the CPH was collected shares boundary with Ekiti State University farms and main campus. Similarly, 30.0 kg of cow dung (CD) was collected from the Liarage in Dutse Abattoir, Jigawa State.

## **2.2. Preparation of Cocoa Pod Husk and Cow Dung**

All the unwanted components; twigs, dried cocoa seeds and leaves in the CPH were taken off so as to give room for effective composting system. The collected CD was equally sorted by ensuring that all unwanted materials; stones and twigs were taken off completely.

## **2.3. Composting System**

Composting of 10.0 kg of fresh Cocoa pod husks and 10.0 kg of Cow dung was done with a view to generating compost from the mixture. The fresh Cocoa pod husks were chopped into small bits of 5.0 cm breadth and length as outlined by Adegunloye and Olorunnusi (2016) and later mixed thoroughly with the Cow dung by pounding until a homogenous mixture was perceived. The mixture was subsequently put in a self-designed compost container and labeled “composted CPH + CD 1:1 w/w”. The mixture was continuously mixed every five days throughout the 20 days composting period as stated by Vishan *et al.* (2017). Change in temperature of the pile was monitored with the aid of a digital thermometer on a daily basis until the last day of composting. The mixture of the compost was done so as to achieve the release of nutrients gradually through

mineralization as reported by Adeleye and Sridhar (2015).

## **2.4. Isolation Procedure for Compost**

### **Degrading Bacteria**

About Ten (10) grams of compost at the depth of 5.0 cm were collected from the compost pile on the last day of composting out of which 1.0 g each was weighed and suspended into three (3) test tubes containing 9.0 ml sterile distilled water in triplicates. The mixture was vigorously shaken for ten minutes to give room for the formation of supernatant. Subsequently, 1.0 ml of the supernatant was introduced into test tubes (Adeleye and Yerima, 2019) containing Mineral Salt Medium (MSM) prepared according to the prescription of Mukred *et al.* (2008). Cycloheximide (0.2 g /l) was added as antibiotic to all the test tubes to avert the growth of fungi (Vishan *et al.*, 2017). The mixture in the test tubes was then shaken vigorously and allowed to settle. The test tubes were subsequently incubated at 37.0 °C for four (4) days for bacterial growth as indicated by broth medium turbidity. Bacterial growth observed after incubation was sub-cultured into three (3) test tubes containing Trypticase Soy broth (TSB) and incubated at 37.0 °C for 48 hours. The TSB was prepared according to the standard procedure described by the manufacturer (Himedia Laboratory Pvt Limited, India). After incubation of the test tubes, further subculturing was carried out using three (3) Trypticase Soy Agar (TSA) plates. The TSA was prepared according to the standard procedure described by the manufacturer

(Himedia Laboratory Pvt Limited, India). The TSA plates were incubated at 37.0 °C for 48 hours, the colonies that developed were observed and recorded. Eight TSA slants containing pure cultures of the bacterial isolates were subsequently prepared and kept for further tests. Eight (8) distinct bacterial isolates (C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>7</sub> and C<sub>8</sub>) on the slants were subsequently picked and transferred to other glass slides to form smears. Gram staining procedure was then carried out according to Olutiola *et al.* (1991).

### **2.5. Biochemical Tests for the Identification of Bacterial Isolates**

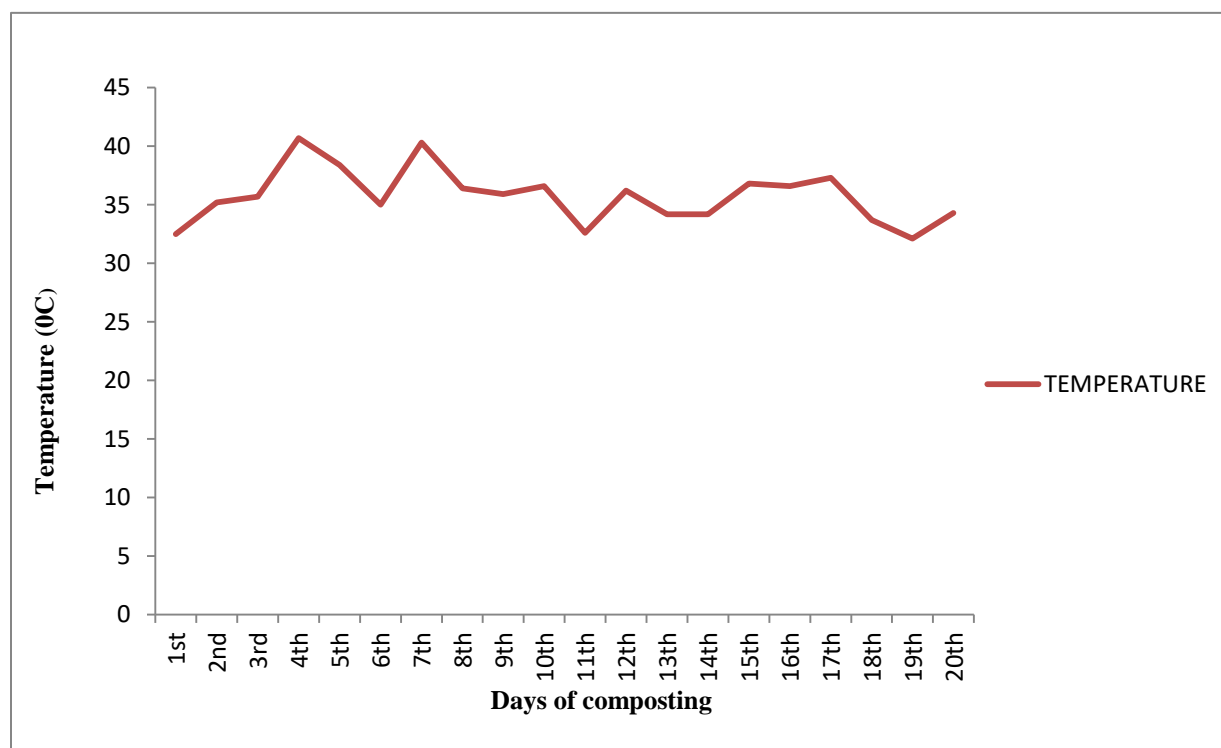
The choices made on the types of biochemical tests conducted on each of the already isolated and gram-stained bacteria in this study were influenced by the procedure prescribed by Barrow and Feltham (1993) for the identification of such groups of bacteria based on their morphological attributes. Biochemical tests were done

according to the procedures established by Cheesebrough (2000); Choopun *et al.* (2002); Ochei and Kolhatkar (2008); Wilson (2012); Hemraj *et al.* (2013); Himedia (2015); Pokhrel (2015); Microbeonline (2019).

## **3. Results and Discussion**

### **3.1 Results**

The composting system set up in this study as shown in recorded varying temperature changes as depicted in Fig. 1. In this study, the lowest temperature (32.7<sup>0</sup>C) of the pile was recorded on the first day while the highest temperature (40.7<sup>0</sup>C) was recorded on the fourth day of composting. The physicochemical properties of the compost generated on the last day of composting are depicted in Table 1. Having conducted gram staining procedure on the distinct colonies that developed and kept on TSA slants, the following results depicted in Table 2 are recorded.



**Figure 1: Change in temperature of the composting system**

**Table 1: Physicochemical Properties of Compost**

Parameters	Compost
Moisture content (%)	2.0
Ash content (%)	65
pH <sub>(water)</sub>	9.45
Organic Carbon (%)	48.25
Total Nitrogen (%)	5.85
Available Phosphorous (mg kg <sup>-1</sup> )	1.48
EC (ds cm <sup>-1</sup> )	8.86
<b>Exchangeable Bases (cmol kg<sup>-1</sup>)</b>	
Potassium	213.16
Calcium	4.8
Magnesium	3.24
Sodium	0.5
CEC	221.7

**Table 2: Gram reactions of distinct bacterial colonies**

Distinct colonies	Gram reactions
C <sub>1</sub>	- rods
C <sub>2</sub>	+ Cocci (sphere)
C <sub>3</sub>	- rods
C <sub>4</sub>	- rods
C <sub>5</sub>	- rods
C <sub>6</sub>	- rods
C <sub>7</sub>	+rods
C <sub>8</sub>	+rods

**KEY:** + = Gram positive, - = Gram negative

Apart from C<sub>2</sub> that was detected to be Gram positive cocci (sphere), all other distinct colonies examined under the microscope recorded Gram negative rods (C<sub>1</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub> and C<sub>6</sub>) and Gram positive rods (C<sub>6</sub> and C<sub>7</sub>) (Table 2). The results of series of biochemical tests conducted in this study are shown in Table 3.

### **3.2. Discussion**

In addition to the thermophiles recorded in the compost pile, the presence of mesophiles that thrive at warmer temperatures between 21 and 32<sup>0</sup>C reported by Patterson (2019) was witnessed in this study. This author implicated these mesophilic bacteria found at this temperature range as the aerobic powerhouses that effect the decomposition that takes in composting process. The generation of heat in compost piles has been reported by Weston (2019). As recorded in this study, this author reported that the activity of the mesophiles gives rise to heat generation and a significant increase in the temperature of the compost which ultimately favours thermophilic organisms that thrive within a temperature range of 40°C and 70

Key: +Positive, =Negative, -Notdone

**Table 2: Biochemical characterization of the bacterial isolates**

Characteristics	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>7</sub>	C <sub>8</sub>
Gram stain	-	+	-	-	-	-	+	+
Cell shape	Rod	Cocci	Rod	Rod	Rod	Rod	Rod	Rod
Nitrate	-	ND	ND	+	ND	ND	-	+
Catalase	ND	ND	ND	ND	ND	ND	ND	ND
Motility	ND	-	+	+	ND	+	+	ND
Oxidase	+	ND	ND	+	ND	ND	-	-
Citrate	+	ND	+	+	ND	-	-	+
Urease	-	ND	+	-	-	-	ND	-
Casein hydrolysis	-	ND	+	ND	+	-	-	ND
Glucose	-	ND	ND	+	+	ND	+	+
Fructose	-	ND	ND	+	ND	ND	ND	ND
Mannose	ND	ND	ND	ND	ND	ND	+	+
Maltose	-	ND	ND	-	ND	ND	ND	ND
Mannitol	-	ND	ND	ND	ND	ND	ND	ND
Sucrose	-	+	+	-	-	-	ND	ND
Xylose	-	ND	-	+	-	+	+	-
Tyrosine hydrolysis	+	ND	ND	ND	ND	ND	ND	ND
Growth on cetrimide agar	+	ND	ND	ND	ND	ND	ND	ND
Indole	ND	ND	-	ND	+	+	-	-
VogesProskauer	ND	-	+	ND	ND	-	+	+
Galactose	ND	ND	ND	ND	ND	ND	-	+
Growth on 10% NaCl	ND	ND	ND	ND	ND	ND	-	+
Methyl Red	ND	ND	-	ND	-	+	ND	ND
Starch	ND	+	-	ND	-	ND	ND	ND
Lactose	ND	+	-	-	-	ND	ND	ND
Phosphatase	ND	+	ND	ND	ND	ND	ND	ND
<b>Possible bacteria</b>	<i>Alcaligenes feacalis</i>	<i>Streptococcus</i> sp	<i>Serratiamarce scens</i>	<i>Pseudomonas</i> sp	<i>Flavobacterium</i> sp	<i>E. coli</i>	<i>Bacillus stearothersophilus</i>	<i>Bacillus subtilis</i>

The results recorded on the microscopic characterization of the bacterial isolates in this study suggest that rod shaped bacteria predominantly contributed to the decomposition of the organic materials composted. It can be seen that all the biochemical tests conducted on C<sub>1</sub> indicated that the isolated bacterium is *Alcaligenes faecalis* while the various biochemical tests conducted to identify C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>7</sub> and C<sub>8</sub> indicated that *Streptococcus* sp, *Serratia marcescens*, *Pseudomonas* sp, *Flavobacterium* sp, *Escherichia coli*, *Bacillus stearothermophilus* and *Bacillus subtilis* respectively. Similar results have been reported by Andrews *et al.* (1994); Alfreider *et al.* (2002); Gentleman *et al.* (2004); Zaidi *et al.* (2009); Partanen *et al.* (2010); Weston (2019).

Interestingly, a similar study conducted by Adegunloye and Olorunnusi (2016) revealed *Bacillus* sp and *Escherichia coli* as the bacteria that had the highest percentage frequency (11.7%) in the pile of compost investigated. The results obtained in this study have revealed that composting is an aerobic process aided by members of facultative anaerobic bacteria involving *Bacillus* sp. Similar assertions have been reported by De Bertolli *et al.* (1980); Vishan *et al.* (2017).

### Conclusion

The method adopted for the isolation of the bacteria responsible for the composting of CPH and CD in this study is effective as eight bacteria were isolated through its adoption. However, the biochemical identification of the bacterial isolates needs

confirmation by subjecting the bacterial isolates into molecular identification.

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