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SHORT COMMUNICATION

Toxin-producing *Escherichia coli* isolates from composting pig waste

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ABSTRACT

Freshly voided excreta of confined pigs were composted using the open heap technique for 40 weeks. At specific intervals, pH and temperature determination as well as isolation and identification of *Escherichia coli* were performed. All *E. coli* isolates recovered were tested for the presence of heat-labile and heat-stable toxins using standard techniques. The values for pH and temperature ranged between 5.8 – 6.9 and 27 °C – 37 °C respectively. A total of 10 (76.92%) out of 13 *E. coli* isolated produced heat-labile toxins while 6 (46.15%) produced heat-stable toxins. All isolates that produced heat-stable toxin also produced heat-labile toxins. Open heap technique should not be encouraged as a method of composting. However, techniques that will ensure temperature of ≥ 55 °C is advocated.

Keywords: Enterotoxigenic *Escherichia coli,* open heap composting technique, pig waste

INTRODUCTION

Compost is the product of aerobic process during which microorganisms play an important role. Essentially, the microorganisms decompose the organic matter into a stable amendment for improving soil quality and fertility (Tiquia, 2005). Microbial quality of the compost reflects the effectiveness of the composting process (Hoffmeister *et al.,* 2005). Therefore a thorough assessment of maturation is crucial because the use of under-matured compost may provoke serious biological damage to soils (Hue and Liu, 1995), along with the proliferation and spreading of potentially pathogenic microorganisms (Hoffmeister *et al.,* 2005). Indeed, Atkinson *et al.* (1996) stressed that the microbial count should be low and should not contain significant quantities of viable pathogenic organisms.

Diarrhoea causing bacteria are mostly implicated in the faecal–oral route of diseases transmission. Many of these bacteria produce toxins that can cause diarrhoea especially enterotoxigenic stains of *Escherichia coli* (ETEC) which are important cause of diarrhoea in infants and adult travellers in the human population. These strains are also responsible for diarrheal diseases of animals such as pig and cattle and may produce types I and II heat-stable and types I and II heat-labile toxins (Hoffmeister *et al*., 2005). Consumption of food and/or water contaminated with ETEC has been reported to be the causes of most diarrhoea out break worldwide (Clarke, 2001). They do this by producing heat-labile or heat-stable toxins or both.

Against this background this study aims to isolate *E. coli* during pig waste composting process and to determine if the *E. coli* isolates produce heat-labile or heat-stable toxins.

MATERIALS AND METHODS

Collection and Processing of Samples

The heaps were not watered at intervals and were not covered both during the day and at night throughout the composing period. These wastes were allowed to compost for 40 weeks at atmospheric temperature of 29 ± 3 °C. At week 0, 1, 2, 4, 6, 8, 10, 14, 18, 20, 24, 30 and 40 the temperature of each composting heap was determined using – 10 °C to 110 °C thermometer. At the above sampling periods, samples were also collected a depth of 30cm from the central surface of every heap and placed inside a sterile universal container, transported to the laboratory in ice packs and processed for pH determination and microbial isolation of *E. coli.*

pH Determination

The pH of each sample was determined using a suspension obtained by dispensing the compost sample in sterile distilled water 1:50 and measured using a pH meter (Electronic Ind. Ltd., England).

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Isolation of *Escherichia coli*

The compost–sterile water mixture prior to pH determination was inoculated on to Eosin–Methylene Blue Agar (EMB, Oxoid, England) and MacConkey Agar (Oxoid England). The plates were incubated at 37 °C for 18 – 24 h. Colonies with greenish metallic sheen on the EMB agar and rose pink colonies on the MacConkey agar were further identified if they were *E. coli.* An organism was identified as *E. coli* if it was a Gram negative bacilli, lactose fermenting, urea negative, citrate negative, motility positive and is indole positive both at 37 and 44 °C (Barrow and Feltham, 2003).

Detection of Heat-Labile Toxin

The ability of the *E. coli* isolate to produce heat-labile toxin was determined using the rabbit ileal–loop method as described previously (Ibeh and Izuagbe 1986; Tsuji *et al.,* 1990). Briefly, adult rabbits were anesthetized with cotton wool soaked in ether in a close vessel. The abdomen of the rabbit was dissected and the ileum brought out and tied into 15 segments using thread. Each loop measured 5.00 cm to another. One loop was used as a positive control and another as a negative control. Using sterile 2 mL needle and syringe, 0.5 mL of overnight broth culture of the *E. coli* isolate in 1% lactase broth was injected with the ileum of each loop. Different syringes and needles were used for each segment. Sterile physiologic saline was injected into the 14th loop as negative control. Known enterotogenic *E. coli* (obtained from Microbiology Department, Veterinary Research Institute Vom, Nigeria) that produce heat-labile toxin, was inoculated into the segment for positive control. The entire ileum was incubated at room temperature for 12 h in sterile normal saline. Volume of fluid accumulated was measured. Fluid accumulation of 0.5 mL and above was regarded as positive for heat labile toxin production.

Detection of Heat-Stable Toxin

The infant mouse dye test as described by Ibeh and Izuagbe (1986) was used to detect the production of heat stable toxin among the *E. coli* isolates. Briefly, the isolates were grown in 1% lactase broth overnight at 37 °C. The overnight broth culture was heated at 60 °C for 30 minutes in a water bath. To 1.0 mL of the supernatant, 2 drops of pontamine sky blue 6BX (Dupont Chemical Co. England) was added. Fourteen infant mice aged 2- 4 days old were allowed to suck their mother's breast. After which, using a 2 mL syringe fitted with 25G hypodermic blunt needle, 0.1 mL of each stained extract was injected intragastrically into each mouse. The 14th mouse was injected with sterile normal saline stained with pontamine sky blue dye and served as negative control. The mice were left at room temperature in glass petri-dishes for 4 h after which they were killed with chloroform. The abdomen of each mouse was dissected and the entire gut length removed and weighed. The rest of the body was also weighted. Ratio of both weighed was determined as shown below.

$$\text{Ratio} = \frac{\text{Gut weight}}{\text{Remaining body weight}}$$

A ratio of 0.085 and above and distention of gut was regarded as positive. The Animal Ethical Committee of the University of Benin approved the protocol for animal use in this study.

RESULTS AND DISCUSSION

The process of composting has been divided into mesophilic, thermophilic and curing stages (Droffner and Yamamoto, 1991). Coliform organisms such as *E. coli* are expected to be killed during the thermophilic stage of composting as temperature ≥ 55 °C are recorded at this stage (Ryckeboer *et al.,* 2003; Tiquia, 2005). Indeed, some workers observed the complete elimination of coliforms before the end of the first four weeks of composting (Tiquia and Tam, 2000). In this study, *E. coli* were isolated throughout the composting period (a total of 13 strains). The isolation of *E. coli* in this study may be due to the low temperature observed, as the temperature ranged from 27 °C to 37 °C (Figure 1). It is important to note that the three stages of composting can only be followed *senso stricto* if heat is not allowed to escape (Kaneshiro *et al.,* 1999). In this study, composting was performed in an open air, which is the method of composting used in this locality. Heat generated can easily be lost to the environment. Hence, the compost temperature never rose to the level that eliminates coliforms.

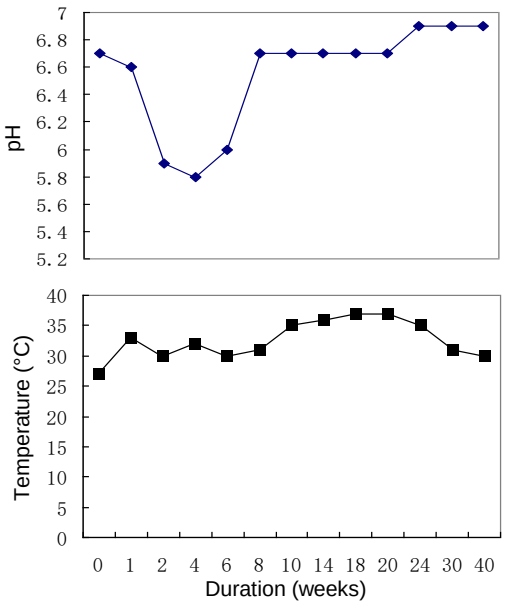

Figure 1: Prevalence of heat-labile and heat-stable enterotoxins among *E. coli* isolate from composting pig waste

The pH decreased initially from 6.7 to 5.8 (from week 0 to week 4). Thereafter, the pH values rose to 6.9 (by the

24th week) and remained unchanged till the end of the composting period (Figure 1). The initial decrease in pH is as a result of metabolic activities of bacterial population which results in the excretion of organic acids (Hoffmeister *et al.,* 2005). As the composting process continues, elimination of carbon dioxide and the degredation of acids and proteins promote the increase in pH values (Hoffmeister *et al.,* 2005). The result from this study indicates that the pH values and temperature recorded throughout the composting period did not affect the survival of *E. coli* in the compost.

A total of 10 (76.92%) out the 13 *E. coli* strains isolated produced toxins. The 10 *E. coli* strains produced heat-labile toxins while 6 of these strains produced heat-stable toxins. The presence of ETEC in compost has earlier been reported (Hoffmeister *et al.,* 2005). This is particularly of concern as most diarrhoea outbreaks occurring world-wide are due to the consumption of contaminated foods and water (Clarke, 2001), and this method of composting is rife in this locality.

The open heap technique used in this study and in this locality, yields under-matured compost, based on the indices studied – temperature, pH and presence of ETEC. This can result in the proliferation and spreading of potentially pathogenic microorganisms (Hoffmeister *et al.,* 2005). It has been recommended that salmonellae and *E. coli* be absent in animal waste compost for use as biofertilizers (Schleiff and Dorn, 1997). Therefore, the practice of open heap composting should be discouraged and other safer techniques should be used to produce compost. However, in the interim, crops harvested from soils enriched with compost produced in the manner described in this paper, should be thoroughly washed with water and salt or properly cooked to eliminate gastroenteric/food poisoning organisms (Schleff and Dorn, 1997). Also good hygiene should be practiced where this kind of organic manure are used, as flies can transfer pathogenic organisms from farm lands to open food or water. This study demonstrated a high prevalence (76.92%) of toxin-producing *E. coli* from composting pig waste. The open heap technique of composting is strongly discouraged. It is important to use techniques, such as closed vessel composting, that will generate enough heat to kill coliforms and other pathogenic microbes.

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