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Occurrence of diversity of bacteria during anaerobic fermentation of cow dung and corn cob

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Abstract
Microbial succession refers to the changing microbial population in a natural or disturbed system. The aim of this study was to isolate and determine bacterial succession in anaerobic fermentation. Cow dung was collected from Oja-Oba in Ekiti State, Nigeria while the corn cob was collected from Ajayi Farms in Akure, Ondo State, Nigeria, and transported to Afe Babalola University (ABUAD) Farms, where it was ground into smaller pieces. One hundred kilograms of cow dung was mixed with water and transferred to digester A, 100 kg of ground corn cob was transferred to digester B and 50 kg each of cow dung and ground corn cob were transferred into digester C. Microorganisms were isolated using pour plate method from day 1 of the fermentation period and at 5 days intervals for the 30 days fermentation period. The bacterial isolates were identified on the basis of their morphological and biochemical characteristics and molecular analysis (DNA extraction, Polymerase Chain Reaction and gene sequencing). Some of the bacteria isolated were Escherichia coli CFT073, Arthrobacter citerus strain NEB577, Klebsiella aerogenes strain AR 0018, Pseudomonas aeruginosa PA01 and Acinetobacter lactucae strain ANC405. Microbial succession of bacteria also showed the dominant organisms belong to Phylum Proteobacteria, followed by phylum Firmicutes and phylum Acinetobacteria. The amount of gas compressed after fermentation in the digesters varied. Digester A- 60kg, Digester B- 12.5kg and Digester C- 64kg with percentage weight of gas of 54%, 50% and 56% respectively. The result from this experiment clearly showed that the anaerobic fermentation of cow dung and ground corn cob involved the interaction between diverse microbial populations at various stages of fermentation.
Keywords: *cow dung, corn cob, anaerobic fermentation, bacterial succession*

INTRODUCTION

Microbial succession occurs within a group of microorganisms as a microhabitat. This type of succession is common in recently disturbed communities or newly available habitat. Microbial communities may also change due to products secreted by the bacteria and fungi present. Changes in pH in a habitat could provide ideal conditions for a new species to inhabit the area. In some cases, the new species evolving may not out-compete the present ones for nutrients leading to the cell death (Franscisco *et al.*, 2015). Bacteria which are simple prokaryotic cells that are found in the environment contain a well-developed cell structure that is responsible for many of their unique biological properties. Under optimal growth conditions, they can grow extremely rapidly and can double as quickly at every 10 minutes (Ogbulie and Nwakanma, 2015).

Huge amount of crop residues and livestock manure are produced every year. However, dealing with these increasing volumes of agricultural wastes is becoming a major problem, because most of the wastes generated are not treated or efficiently recycled (Huang *et al.*, 2013; Renaud *et al.*, 2017). Notably, untreated cow manure has caused serious environmental pollution and this is inimical to human health and the general wellbeing of a community. Thus, there is a need for effective management of these agricultural wastes. Composting is considered to be an effective method for disposal of agricultural solid waste and the final product is suitable for agricultural and horticultural use as part of a sustainable strategy (Kang *et al.*, 2014; López-González *et al.*, 2015).

Materials and methods

Samples collection

Samples of corn cob were collected from Ajayi Farms in Akure, Ondo State in January 2020 and cow dung was collected from Oja Oba in Ado-Ekiti, Ekiti State, Nigeria. The corn cob was milled into smaller sizes using grinding machine (M6FFC60) from Afe Babalola University (ABUAD) Farm to reduce their sizes and increase the surface area of the wastes for faster and optimum degradation. The substrate was transported to the Microbiology Laboratory, Afe Babalola University, Ado-Ekiti in polythene bags for the identification of organisms involved in the fermentation process.

Anaerobic Digestion of Substrates

The experiments were conducted in fabricated digesters. One hundred kilograms of cow dung was fed into digester (A), one hundred kilograms of ground

corn cob was fed into a digester (B) and one hundred kilograms of a mixture of cow dung and ground corn cob was fed into digester (C) in a ratio 1:1 through the inlet. The inlet and outlet were blocked to ensure anaerobic condition. The fermentation lasted for thirty (30) days and organisms were isolated from the digesters on day 1 and every 5 days. After 30 days, the gas produced from each of the digesters were compressed into different gas cylinders according to the modified method described by Jyothillakshmi and Prakash (2016).

Isolation, characterization and identification of bacteria

Isolation of indigenous bacteria was carried out using the ground corn cob waste, cow dung and a mixture of the substrate before fermentation, during fermentation and after fermentation for a period of 40 days. One gram of the substrate was added into 9 ml of sterile distilled water in a test tube and mixed properly. Ten-fold serial dilutions were carried out and the desired dilutions were plated using pour plate technique. The poured plates were incubated at 35^{o}C for 24 h. Nutrient agar and MacConkey agar were used. The bacterial isolates were identified on the basis of their morphological, and biochemical characteristics and molecular analysis (DNA extraction, Polymerase Chain Reaction and gene sequencing) (Tankeshwar, 2015).

Bacteria DNA Extraction and Purification

This procedure was carried out using Zymo research fungal/bacterial DNA miniprep kit. Isolates used were cultured in broth overnight. The broth was transferred to a ZR bashing beads where ™lysis solution was added to the broth. The tube was centrifuged in a micro centrifuge at 10,000rpm for one minute. Four hundred 400 µl of the supernatant was transferred to a Zymo-Spin ™IV spin filter in a collection tube and centrifuged at 7000rpm for one minute. 1,200 µl of bacterial DNA binding buffer was added to the filtrate in the collection tube, then 800 µl of the mixture was transferred to a Zymo-Spin ™11C column in another collection tube and centrifuged at 10000xg for 1 minute . 500 µl bacterial DNA wash Buffer was added to the Zymo-spin ™IIC column and centrifuge at 10,000 kg for 1 minute. The Zymospin ™IIC column was transferred to a clean 1.5ml microcentrifuge tube and 100 µl DNA Elusion Buffer was added directly to the column matrix. It was centrifuge at 10000rpm for 30 seconds to elute the DNA. Finally, the spin column was discarded and the final product was subjected to 1% agarose gel electrophoresis and NanoDrop Machine (Applied Biosystem, 2010).

Polymerase Chain Reaction Amplification

An ependorff tube was prepared with the following mix: 77.5 µl of water was added followed by 10.0 µl of 10x buffer, 6.5 µl of Magnesium chloride $(MgCl_2)_2$ one µl microliter of primer mono A (16SF: GTGCCAGCAGCCGCGCTAA), + 1 µl of mono B (16SF:AGACCCGGGAACGTATTCAC), 2 µl of dNTP mix, 1 µl of chromosomal DNA and 2.5 µl of Taq polymerase. The contents were mixed thoroughly and 5 µl each were transferred into new tubes and the following processes happened in a thermocycler machine: denaturation at 94^{o}C for 5 minutes and subsequently for 30 seconds. Extension at 72^{o}C for 45 seconds: above processes occurred in 36 cycles and final extension was at 72^{o}C for 7 minutes. The products were further purified by adding 2 volumes (20 µl) of absolute ethanol, incubated at room temperature for 15 minutes and then centrifuged at 10,000rpm for 15 minutes. Supernatant was discarded and the product was air dried. The final product was held at 10^{o}C for further analysis (Kumar *et al.*, 2016).

Concentration of DNA

Using a Nanp drop ND1000 (Thermo Scientific, USA) mavjine, DFNA was quantified by calibrating the machine by 1 µl of water, followed by one µl microliter blank (Tri-EDTA buffer) and then 1 µl of DNA sample to be quantified (Reeza *et al.*, 2012).

Gel electrophoresis

Twenty µl each of the PCR product was pipetted into 5 µl of bromophenylene dye (tracking dye) and mixed well, then layered into wells of 1% agarose gel and subjected to electrophoresis. The gels were stained with ethidium bromide for 5 minutes, drained and photographed under ultra violet light and various migration molecular weights of the DNA photographed were recorded (Applied Biosystems, 2010).

Gene sequencing

The amplicons from the polymerase chain reaction were subjected to sequencing reaction using BigDye Terminator v 3.1 Cycle Sequencing Kit, following manufacturer's guidelines. The products were loaded into 3130 xl Genetic Analyser to generate the molecular sequences of each amplicon (Applied Biosystems, 2010).

Base Sequence Analysis

The base sequences generated from amplicon were analysed by a combination of Basic Local Alignment Search Tool (BLAST) and Bio-edited. Sequences were submitted as query at *http://www.ncbi.nm.nih.gov/Blast,cg*i for comparison with database sequences using NCBI nucleotide BLAST. Isolates were identified based on DNA-DNA similarity at 95%.

Phylogenetic Analysis

Phylogenetic analysis was carried out using Align by ClustalW method based on the partial sequences of 16s rRNA gene representative isolates in this study. Isolates in this study were compared to Neighbor-joining in molecular evolutionary genetics analysis software (MRGA 7) with known reference strain from NCBI database. The best substitution model that described the confidence interval of the resultant tree was selected (Garcia-Sepulveda *et al.*, 2010).

Evolutionary Relationship of Taxa

The evolutionary tree was inferred using the Neighbour-joining tree method. The percentage of replicate tree for which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is done to scale, with branch lengths in the same unit as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 18 nucleotide sequences. All position containing gaps and missing data were eliminated. Evolutionary analysis was conducted in MEGA 7 (Chun and Rainey, 2014).

Statistical Analysis

all treatments were done in triplicates. The significant difference of the results was evaluated using multiple range tests with SPSS version 20 software. For all the tests, the significance was determined at the level of $P<0.05$.

RESULTS

Bacteria were isolated from cow dung, corn cob and a mixture of cow dung and corn cob.

Table 1 showed the succession and frequency of occurrence in bacterial isolates in digester A containing cow dung. On the first day before fermentation began, twelves isolates were identified from the digester. The bacterial isolates included *Pseudomonas* sp, *Klebsiella* sp, *Serratia* sp, *Staphylococcus* sp, *Lactobacillus* sp, *Proteus* sp, *Micrococcus* sp, *Clostridium* sp, *Salmonella* sp, *Streptococcus* sp, *Arthrobacter* sp and *Citrobacter* sp.

Between day 5 and day 10, organisms such as *Pseudomonas* sp, *Klebsiella* sp, *Enterobacter* sp, *Bacillus* sp, *Serratia* sp, *Staphyloccoccus* sp, *Shigella* sp, *Yersina* sp, *Proteus* sp, *Micrococcus* sp, *Corynebacterium* sp, *Escherichia* sp, *Streptococcus* sp all appeared. Isolate D1 was present during the 30 day fermentation period with percentage of occurrence of 100%. The probable organism was Pseudomonas. Also, C4 and C6 had a 100% frequency of occurrence and the probable organisms were Bacillus sp and Staphylococcus sp respectively. The isolates with the least frequency of occurrence of 14.29% were CD 13 and CD 18. CD 13 was present on Day 5 while CD 18 was present at Day 10 of the fermentation period. The probable organisms were *Corynebacterium* sp and *Janibacter* sp respectively. Next to these were isolates that showed a frequency of occurrence of 28.57% and they were CD 5, CD 8, CD 12 and CD 17. The probable organisms were *Serratia* sp, *Lactobacillus* sp, *Clostriduim* sp and *Arthrobacter* sp respectively and each appeared twice during the fermentation period.

Table 2 showed the bacterial succession and frequency of occurrence of isolates in digester B. A total of twenty one (21) organisms were identified from the substrates before fermentation. The bacterial isolates included *Pseudomonas* sp, *Klebsiella* sp, *Enterobacter* sp, *Proteus* sp, *Micrococcus* sp, *Corynebacterium* sp, *Escherichia* sp, *Acinetobacter* sp and *Serratia* sp. Isolate CB 4 was present through the period of fermentation with frequency of occurrence of 100% and the probable organism was *Bacillus* sp. The isolates with the next highest frequency of occurrence of 71.42% were CB 12 and CD 13. The probable organisms were *Micrococcus* sp and *Clostridium* sp. CB 12 was present on Days 1, 5 and 10, absent on Day 15 and 20 and reappeared on Day 25 and 30 while CD 13 was present from Day 10 to the end of the fermentation period. The isolates with the least frequency of occurrence of 14.29% were CB 10, CB 16, CB 18, CB 20 and CB 21. CB 10 and CB 14 were present only on day 1. CB 18 was present on Day 5 while CB 20 and CB 21 were present only on Day 10.

Table 3 showed the succession and frequency of occurrence of bacterial isolates in digester C containing the mixture of both cow dung and corn cob. A total of nineteen organisms were isolated at inception. The organisms isolated include *Pseudomonas* sp, *Klebsiella* sp, *Bacillus* sp, *Serratia* sp, *Salmonella* sp, *Shigella* sp, *Lactobacillus* sp, *Yersinia* sp, *Micrococcus* sp, *Clostridium* sp, *Corynebacterium* sp, *Escherichia* sp, *Citrobacter* sp, *Acinetobacter* sp, *Providencia* sp, *Morgannella* sp. Isolates M1, M2 and M4 had frequency of occurrence of 100% that is, they were present during the 30 days fermentation period. The probable organisms were *Pseudomonas* sp, *Klebsiella* sp and *Bacillus* sp respectively. Isolate M15 had a frequency of 85.71% and the probable organism was *Escherichia coli* sp. On Day 30, this organism was absent. It was only one isolate, M18, that had frequency of occurrence of 14.29% and the probable organism was *Providencia* sp which appeared on Day 1 of the fermentation period.

Table 1: Succession of bacterial isolates and frequency of occurrence in digester A containing cow dung

Sample codes	Days							Probable organism	Percentage Occurrence (%)
	1	5	10	15	20	25	30		
CD1	+	+	+	+	+	+	+	*Pseudomonas* sp	100.00
CD2	+	+	+	-	-	+	+	*Klebsiella* sp	71.42
CD3	-	+	+	+	+	-	-	*Enterobacter* sp	57.14
CD4	+	+	+	+	+	+	+	*Bacillus* sp	100.00
CD5	+	+	-	-	-	-	-	*Serratia* sp	28.57
CD6	+	+	+	+	+	+	+	*Staphylococcus* sp	100.00
CD7	+	+	-	-	+	-	-	*Shigella* sp	42.85
CD8	+	+	-	-	-	-	-	*Lactobacillus* sp	28.57
CD9	-	+	+	+	-	-	-	*Yersinia* sp	42.85
CD10	+	+	+	-	-	-	-	*Proteus* sp	42.85
CD11	+	+	+	+	-	-	-	*Micrococcus* sp	57.14
CD12	+	-	-	-	-	-	+	*Clostridium* sp	28.57
CD13	-	+	-	-	-	-	-	*Corynebacterium* sp	14.29
CD14	-	+	+	+	+	+	+	*Escherichia* sp	85.75
CD15	+	-	-	+	+	-	-	*Salmonella* sp	42.85
CD16	+	+	+	-	-	-	-	*Streptococcus* sp	42.85
CD17	+	-	-	+	-	-	-	*Arthrobacter* sp	28.57
CD18	-	-	+	-	-	-	-	*Janibacter* sp	14.29
CD19	+	+	+	+	-	-	-	*Citrobacter* sp	57.14

Key
CD =Cow dung
+= Present
-= negative

Table 2: Succession of bacterial isolates and frequency of occurrence in digester B containing ground corn cob

Sample codes	Days							Probable Organism	Percentage Occurrence (%)
	1	5	10	15	20	25	30		
CB1	+	+	-	+	+	-	-	*Pseudomonas* sp	57.14
CB2	+	+	-	+	+	-	-	*Klebsiella* sp	57.14
CB3	+	-	-	+	+	+	-	*Enterobacter* sp	57.14
CB4	+	+	+	+	+	+	+	*Bacillus* sp	100.00
CB5	+	+	+	-	-	-	-	*Serratia* sp	42.85
CB6	-	+	+	+	-	-	-	*Staphylococcus* sp	42.85
CB7	+	+	-	-	-	-	-	*Salmonella* sp	28.57
CB8	+	+	-	-	-	-	-	*Shigella* sp	28.57
CB9	+	+	+	-	-	-	-	*Lactobacillus* sp	42.85
CB10	+	-	-	-	-	-	-	*Yersinia* sp	14.29
CB11	+	+	+	-	-	-	-	*Proteus* sp	42.85
CB12	+	+	+	-	-	+	+	*Micrococcus* sp	71.42
CB13	-	-	+	+	+	+	+	*Clostridium* sp	71.42
CB14	+	+	+	-	-	-	-	*Corynebacterium* sp	42.85
CB15	+	+	+	+	-	-	-	*Escherichia* sp	42.85
CB16	+	-	-	-	-	-	-	*Acinetobacter* sp	14.29
CB17	-	+	+	-	-	-	-	*Aeromonas* sp	28.57
CB18	-	+	-	-	-	-	-	*Planococcus* sp	14.29
CB19	-	+	+	+	-	-	-	*Citrobacter* sp	42.85
CB20	-	-	+	-	-	-	-	*Streptococcus* sp	14.29
CB21	-	-	+	-	-	-	-	*Providencia* sp	14.29

Key
CB= corn cob
+ = Positive
- = Negative

Table 3: Succession of bacterial isolates and frequency of occurrence in digester C containing the mixture of ground corn cob and cow dung

Sample codes	Days							Probable Organism	Percentage Occurrence (%)
	1	5	10	15	20	25	30		
M1	+	+	+	+	+	+	+	*Pseudomonas* sp	100.00
M2	+	+	+	+	+	+	+	*Klebsiella* sp	100.00
M3	-	+	+	+	+	+	-	*Enterobacter* sp	71.42
M4	+	+	+	+	+	+	+	*Bacillus* sp	100.00
M5	+	+	+	-	-	-	-	*Serratia* sp	42.85
M6	-	+	+	-	-	+	+	*Staphylococcus* sp	57.14
M7	+	-	+	+	-	-	-	*Salmonella* sp	42.85
M8	+	-	-	+	-	-	-	*Shigella* sp	28.57
M9	+	+	+	+	-	-	-	*Lactobacillus* sp	57.14
M10	+	-	-	+	-	-	-	*Yersinia* sp	28.57
M11	-	+	+	+	-	-	-	*Proteus* sp	57.14
M12	+	+	+	-	-	-	+	*Micrococcus* sp	57.14
M13	+	+	-	-	-	+	+	*Clostridium* sp	57.14
M14	+	+	+	-	-	-	-	*Corynebacterium* sp	42.85
M15	+	+	+	+	+	+	-	*Escherichia* sp	85.71
M16	+	+	+	-	-	-	-	*Citrobacter* sp	42.85
M17	+	+	+	-	-	-	-	*Acinetobacter* sp	42.85
M18	+	-	-	-	-	-	-	*Providencia* sp	14.29
M19	+	+	+	-	-	-	-	*Morganella* sp	42.85

Key
M= Mixture of corn cob and cow dung
+ = Positive
- = Negative

Table 4 showed the BLAST hits of microorganisms isolated from the digesters during the thirty days fermentation. The organisms identified in digester A were *Micrococcus terreus* strain FeSTB0-8, *Proteus mirabilis* HI4320 strain, *Salmonella enterica* subsp. enterica serovar Typhi Ty2 and *Klebsiella aerogenes strain* AR 0018. Molecular analyses of the isolates in digester (B) showed that the organisms are *Bacillus cereus* strain FDAARGOS 797, *Arthrobacter citerus* strain NEB577 and *Sphigobium lactosutens* strain XWAA3417. The organisms isolated in digester C were *Pseudomonas aeruginosa* PA01, *Acinetobacter lactucae* strain ANC405, *Enterobacter hormaechei* strain 0992-72, *Clostridium sartagoforme* strain AAUI and *Escherichia coli* CFT073.

Figure 1 showed the phylogenetic relationship among the isolates organisms. The phylogenetic tree had four clades. The first clade belonged to three organisms namely, *Sphigobium Lactosutens* strain XWAA3417, *Bacillus cereus* strain FDAARGOS 797 and *Acinetobacter lactucae* strain ANC405. *Sphigobium lactosutens* strain XWAA3417 and *Bacillus cereus* strain FDAARGOS 797 are sister taxons with equal tree length between them. However, they are distancely related to *Acinetobacter lactucae* strain ANC405.

The second clade consisted of Proteus mirabilis HI4320 strain and *Pseudomonas aeruginosa* PA01. *Proteus mirabilis* HI4320 strain had a longer branch chain of 0.6 which was higher than *Pseudomonas aeruginosa* PA01 of branch length 0.2.

The third clade consisted of three organisms.They were, *Micrococcus terreus* strain FeSTB0-8, *Clostridium sartagoforme* strain AAUI and *Enterobacter hormaechei* strain 0992-72. They all shared a common ancestor. *Micrococcus terreus* strain FeSTB0-8 had a longer branch length from its ancestor. On the other hand, C*lostridium sartagoforme* strain AAUI and *Enterobacter hormaechei* strain 0992-72 were more closely related.

Salmonella enterica subsp. enterica serovar Typhi Ty2, *Escherichia coli* CFT073, *Arthrobacter citerus* strain NEB577 were *Klebsiella aerogenes* strain AR 0018 belonged to the fourth clade. *Salmonella enterica* subsp. *enterica serovar* Typhi Ty2 and *Klebsiella aerogenes* strain AR 0018 were closely related. *Escherichia coli* CFT073 and *Arthrobacter citerus* strain NEB577 are also closely related with the same branch length of 0.5. *Arthrobacter citerus* strain NEB577 and S*almonella enterica* subsp. enterica serovar Typhi Ty2 were distancely related with 0.7 branch length.

Table 4: BLAST hit of representative isolates

Sample code	Percentage identity	Organism	Accession no
CB4	93.87	*Bacillus cereus* strain FDAARGOS 797	NZ_ CP053931.1
M15	95.35	*Escherichia coli* CFT073	AE014075.1
M13	97.78	*Clostridium sartagoforme* strain AAUI	KC433939
CD 11	92.64	*Micrococcus terreus* strain FeSTB0-8	LT718606.1
M3	94.89	*Enterobacter hormaechei* strain 0992-72	NR 042154.1
CD 10	95.83	*Proteus mirabilis* HI4320 strain	NR 075212.1
CD17	92.00	*Arthrobacter citreus* strain NEB577	NZ CP053688.1
CB22	97.70	Sp*higobium lactosutens* strain XWAA3417	NZ_ QJQX01000071.1
M17	97.79	*Acinetobacter lactucae strain* ANC405	NZ_ KB976990.1
M1	84.29	*Pseudomonas aeruginosa* PA01	NC_002516.2
CD15	98.87	*Salmonella enterica* subsp. enterica serovar Typhi Ty2	NC 003197.2
CD2	92.80	*Klebsiella aerogenes* strain AR 0018	CP024880.1

Key

CD10, CD2, CD11, CD15, CD17 - Isolates from digester A (cow dung)

CB4, CB22 – Isolates from digester B (corn cob).

M1, M3, M13, M15, M17- Isolates from digester C (mixture of cow dung and corn cob).

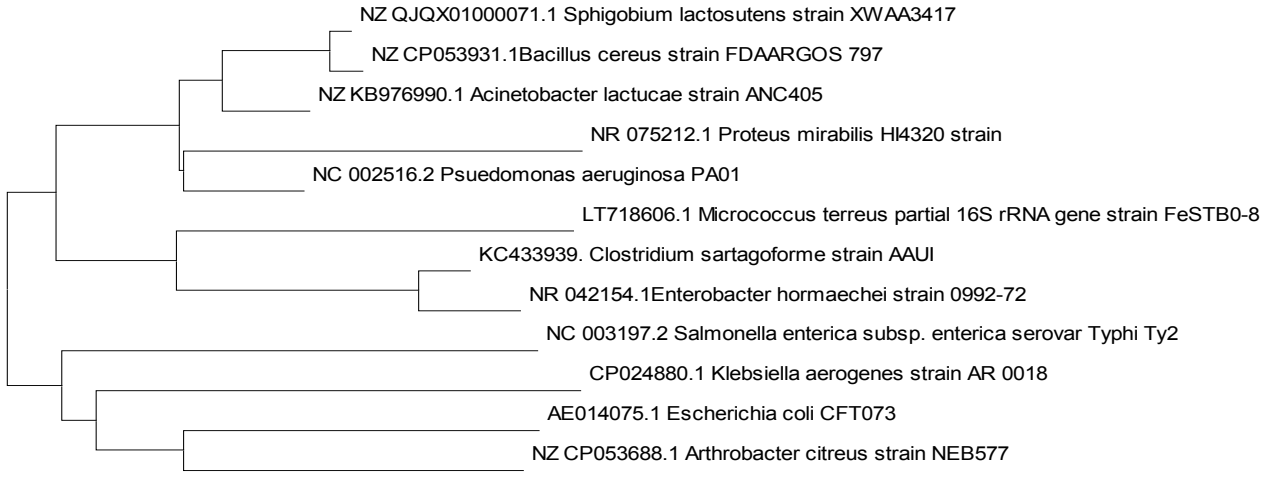

Figure 1: Phylogenetic tree among representative bacterial isolates.

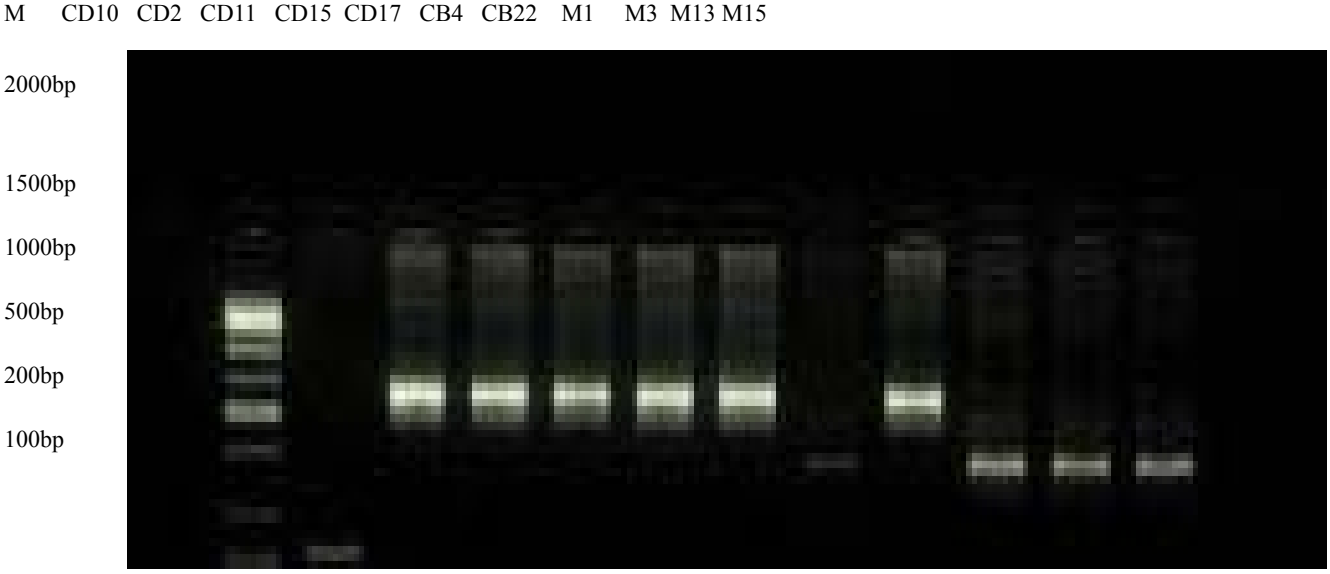

Plate 1: Ethidium-bromide stained agaarose gel after polymerase chain reaction of representative isolates

Key
CD10, CD2, CD11, CD15, CD17 - Isolates from digester (A) containing cow dung
CB4, CB22 – Isolates from digester (B) containing corn cob.
M1, M3, M13, M15, M17- Isolates from digester (C) containing mixture of cow dung and corn cob

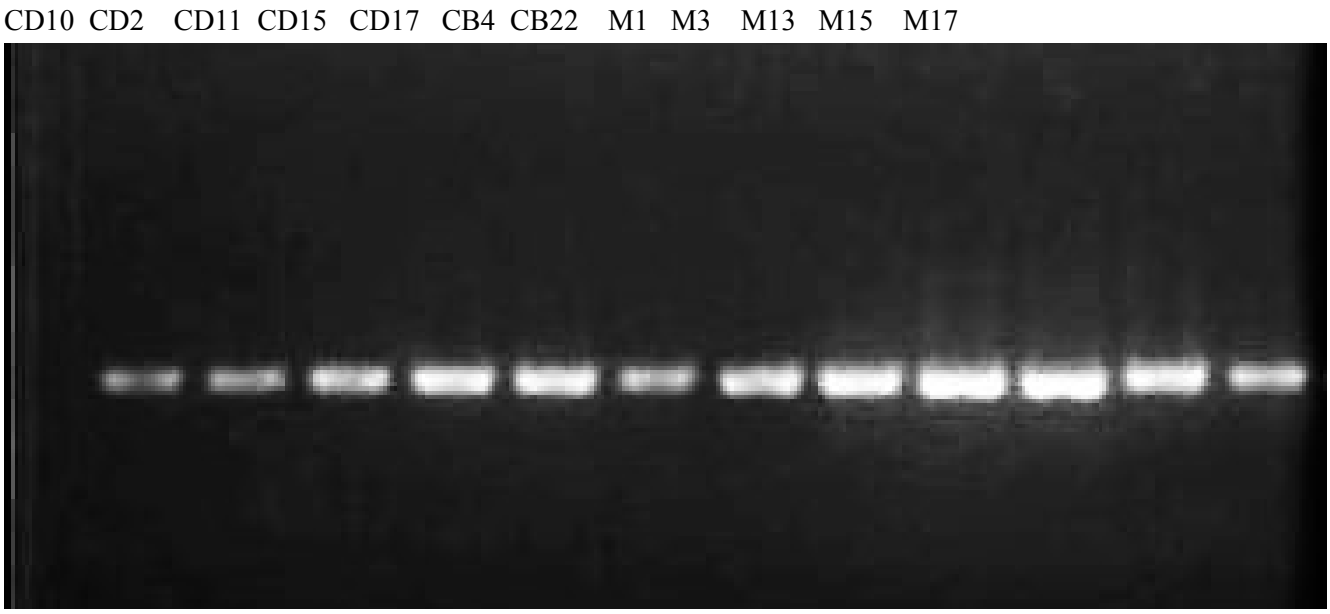

Plate 2: Deoxyribonucleic acid of representative isolates.
Key
CD10, CD2, CD11, CD15, CD17 - Isolates from digester A (cow dung).
CB4, CB22 – Isolates from digester B (corn cob).
M1, M3, M13, M15, M17- Isolates from digester C (mixture of cow dung and corn cob).

Table 5 showed the weight of gas produced from the three digesters. The weight of empty cylinders used for digester A was 50 kg, while 12.5 kg for digester B and 50 kg for digester C. A total weight of 110 kg with the gas was realized from digester A, 25 kg was realized from digester B and 114 kg in digester C. Digester C produced the highest amount of gas - 64kg, followed by digester A with 60 kg and digester B was 12.5 kg.

Table 5: Weight of gas produced at the end of the 30 days fermentation period.

Digester	Weight of empty cylinder (kg)	Weight of cylinder with gas (kg)	Weight of gas (kg)	Percentage weight of gas (%)
A	50	110	60	54
B	12.5	25	12.5	50
C	50	114	64	56

Key

A= Cow dung

B= Corn cob

C= Mixture of cow dung and corn cob.

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DISCUSSION

Anaerobic fermentation of cow dung and ground corn cob was carried out in digesters singly and as a mixture and bacteria were isolated and identified and the succession of these isolates as the fermentation progressed over a period of 30 days was also investigated.

The result from this study showed that certain species of bacteria appeared to extend over one stage of the fermentation period to another, suggesting a succession in species of anaerobic bacteria during the process of biogas production. Similar results were also observed by Asikong *et al* (2016) in the study carried out on microorganism associated with biogas production using vegetable (*Telfaira occidentalis*) wastes, banana peels and pig dung as substrates. Microbiological analyses were carried out on the substrates to determine the presence of bacteria and also their succession in the digesters. Some of the organisms isolated on day 1 (before fermentation) were the species of *Pseudomonas, Klebsiella, Enterobacter, Bacillus, Shigella, Lactobacillus, Yersina, Proteus , Micrococcus, Escherichia* and *Lactobacillus*. Some organisms isolated in this study such as *Proteus, Lactobacillus* and *Pseudomonas* sp were shown to be associated with agricultural system such as corn cob. These results were supported by study carried out by Ekundayo *et al.*, (2011) who isolated *Lactobacillus*, *Proteus, Micrococcus, Klebsiella, Providencia and Clostridium* sp and *Bacillus subtilis* from maize husks and maize cob.

Species of *Klebsiella* and *Bacillus* were isolated from the first day to day 20 of fermentation in digester C. The major bacterial isolates obtained during the 30 days period largely belonged to the phylum Firmicutes, Proteobacteria and Actinobacteria. According to Zhou *et al* (2018), Phylum Bacteroides, Proteobacteria and Firmicutes represented more than 90% of all the sequences identified in their study. Mesophilic organisms dominated the initial decomposition process by releasing heat from the breakdown of the large amount of easily degradable organic matter Asikong *et al.* However, several such as the increase and decrease of temperature affected microorganisms communities. (Huhe *et al.*, 2017). The presence of Firmicutes and other group of phylum in the system increased as the digestion of substrates went on. This may be due to the presence of sugars and other materials that are easily degradable which in turn supplied enough nutrients through the decomposition process, as similarly reported by Liu *et al.*, (2017).

The biogas production process from each substrate and its co-digestion were investigated under this study. During the first four days of fermentation, there was no gas produced from the three digesters, this can be explained to be due to the metamorphic growth process of microorganisms. Akindele *et al* (2018) also observed similar results where they recorded no gas production in the digesters used during the first four days during the fermentation of corn cobs, pigs and poultry drippings. While biogas production started on the 5th day in digester A and digester C, it started on the 8th day for digester B (ground corn cob). There was a notably increase in gas production as the retention time increased. This is largely due to the presence and increased growth/numbers of microorganisms, which led to an increase in fermentation products that eventually gave rise to biogas production. Digester C which contains mixtures of cow dung and ground corn cob produced 60kg of gas, followed by digester A which contained cow dung with 60 kg and 12.5 kg in digester B with ground corn cob. Digester C produced the highest amount of gas, this may be as a result of the combination of combined substrates were used during its fermentation process which gave a wider community of microbes.

The higher biogas yield for the combined substrate is in agreement with a similar work by Kanger (2013) which reported that co-digestion of food waste with sewage sludge resulted in higher biogas yields when with organic materials are converted biogas with organic fertilizer (effluents) as bio-products. These are end products of biogas technology unlike simple composting which produces only fertilizers from

organic solid wastes. Methane is the main constituent of biogas. About 90% of energy of substrate is retained in methane. On the other hand, digester B with ground corn cob substrate contained lignin with other cellulosic materials thereby hindering the production of gas. Lignin is not easily degradable by microorganisms resulting in longer period of lag phase.

CONCLUSION

This finding gave an insight to the understanding of the succession of microbial communities in cow dung and corn cob composting and also, the amount of gas that can be produced during anaerobic fermentation.

RECOMMENDATION

Wastes from agricultural practices such as cow dung and corn cob which constitutes environmental pollution can be made to undergo anaerobic fermentation and converted to biogas and energy thereby getting rid of pollutants in the environment.

CONFLICT OF INTEREST

None

REFERENCES

Akindele, O., Kamoru, B. and Olusola, A. (2018). Biogas production from anaerobic co-digestion of corn cob, pigs and poultry drippings. ABUAD *Journal of Engineering Research and Development*. 1:273-282

Ekundayo, A., Adetuyi, F. and Ekundayo, F. (2011). In vitro antifungal activities of bacteria associated with maize husks and cobs. *Research Journal of Microbiology*. 6: 418-424.

Franscisco, D., James, S., Elsas, D. and Joana, F. (2015). Disentangling mechanisms that mediate the balance between balance stochastic and deterministic processes in microbial succession. *Proceedings of the National Academy of Sciences of the United States of America*.112: 1326-1332.

Huang, H., Geng, G., Yu, X. and Liu, Y. (2013). The state of processing in intensive dairy farm. *China Dairy Cattle*. 1:46–48.

Huhe, J., Wu, Y. and Cheng, Y. (2017). Bacterial and fungal communities and contribution of physicochemical factors during cattle farm waste composting. *Microbiology Open*. 6:e00518.

Kang, W., Kim, I. H., Lee, T. J., Kim, K. Y. and Kim, D. (2014). Effect of temperature on bacterial emissions in composting of swine manure. *Waste Management*. 34: 1006 – 1011.

Kanger, K. (2013). Biogas production under co-digestion of food waste with sewage sludge (Doctoral dissertation, Tartu Ülikool).

Liu, L., Wang, S., Guo, X., Zhao, T. and Zhang, B. (2017). Succession and diversity of microorganisms and their association with physicochemical properties during green waste thermophilic composting. *Waste Management*. 73:101–112.

López-González, J. A., Suárez-Estrella, F., Vargas-García, M. C., Jurado, M. J. and López-Moreno, J. (2015). Dynamics of bacterial microbiota during lignocellulosic waste composting: studies upon its structure, functionality and biodiversity. *Bioresource Technology*. 175: 406–416.

Ogbulie, T.E. and Nwakanma, C. (2015). Microorganisms and man In: Essential Analytical Methods in Biotechnology. WEBSmedia communication, Nigeria, p. 1-28.

Renaud, M., Chelinho, S., Alvarenga, P., Mourinha, C., Palma, P. and Sousa, J. P. (2017). Organic wastes as soil amendments - effects assessment towards soil invertebrates. *Journal of Hazardous Material*. 330:149–156.

Sambrook, J., Maccallum, P. and Russell, D. (2001). Molecular Cloning: A Laboratory Manual 3rd. Cold Spring Harbor Press, New York.

Zhang, L., Zhang, H., Wang, Z., Chen, G. and Wang, L. (2016). Dynamic changes of the dominant functioning microbial community in the compost of a 90- m3 aerobic solid state fermentor revealed by integrated metaomics. *Bioresource Technology*. 203: 1–10.

Zhou, H., Gu, W., Sun, W. and Hay, A. G. (2018). A microbial community snapshot of windrows from a commercial composting facility. *Applied Microbiology Biotechnology.* 102; 8069 – 8077

Chun, C. and Leigh, A. (2014). Metabolic versatility in methanogens. *Current Opinion in Biotechnology.* 29:70-75.

Garcia-Sepulveda, C., Carrillo-Acuna, E. and Barrigo-Moreno, M. (2010). Maxiprep genomic DNA extraction for molecular epidemiology studies and biorepositors. *Molecular Biology Reports.* 37:1883-1890.

Chun, J. and Rainey, F. (2014). Integrating genomics into the taxonomy and systematics of bacteria and archaea. *International Journal of Systematic and Evolutionary Microbiology.* 64:316-324.

Applied Biosystem (2010). BigDye Terminator v3.1 cycle sequencing kit protocol. Available (online) at www.appliedbiosystems.com

Reeza, M., Hamid, B., Mehrdad, M., Samanesh, K. and Fatemah, S. (2012). Rapid DNA extraction of bacterial genome using laundry detergents and assessment of the efficiency of DNA downstream process using polymerase chain reaction. *African Journal of Biotechnology.* 11:173-178.

Kumar, S., Stecher, G. and Tumura, K. (2016). MEGA 7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution.* 33:1870-1874.