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Characterization of bacteria isolated from rumen substrates of goat (*Capra aegagrus hircus*)

Odeyemi, A. T.[1]*, Oluwole, O. A.[2] and Oso, M. O[3]

[1]Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria. E-mail: adebowaleodeyemi@gmail.com
[2]Department of Science Laboratory Technology, Ekiti State University, Ado-Ekiti, Nigeria. E-mail: solaoluwole@ymail.com
[3]Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria. E-mail: moyomade1@gmail.com

Abstract

Studies on microorganisms associated with rumen substrates of goat (*Capra* aegagrus hircus) were done through aerobic and anaerobic fermentation for a period of 12 days. Microscopic, biochemical and molecular identification were carried out. Also proximate values of the substrates over the period of fermentation were analyzed. Seven (7) aerobic bacteria species were isolated, viz.; *Bacillus* spp, *Escherichia coli*, *Lactobacillus* spp, *Proteus* spp, *Pseudomonas* spp, *Staphylococcus* spp, and *Serratia* spp, with *Pseudomonas* spp having the highest percentage of occurrence of 30.7%, followed by *Bacillus* spp (23.7%), followed by *Lactobacillus* spp, *Shigella* spp, *Proteus* spp, *Staphylococcus* spp, having occurrence of 7.6% each. Also, five (5) anaerobic bacteria were also isolated of which *Pseudomonas* spp was dominant with 33.3%, *Bacillus* spp and *Lactobacillus* spp had occurrence of 25%, *Serratia* spp, *Escherichia* spp had least occurrence of 8.3% each. There was increase in some vital proximate values due to the biodegradability nature of the rumen normal flora for the period of fermentation. As a result of this, two bacterial isolates (aerobic and anaerobic) which recorded the highest microbial load and mineral level on the 12th day were characterized by sequence analysis of 16S ribosomal RNA gene. The aerobic isolate was discovered to be 99% identical to *Bacillus anthracis* CGS-1strain while the anaerobic isolate was identified as *Pseudomonas aeruginosa* N17.35strain. The importance of the characterized microbes in improving the proximate value of utilized fermented rumen content is discussed.

Keywords: Rumen substrates, Goat, Aerobic bacteria, Fermentation, 16S ribosomal RNA.

1. Introduction

The rumen is an ideal microbial habitat because the conditions that exist are conducive for the survival and growth of microorganisms (Krehbiel *et al.,* 2003). The water drank by the animal and the saliva which is exocrine secretion received by the rumen, provide a moist environment required for microbial growth (Krause *et al.,* 2003). The ingested food provides nutrients needed for microbial growth and activity. Normal reticulo-ruminal motility (peristalsis and antiperistalsis) helps mix the contents, which brings microbes into contact with fresh substrate. Since the environment inside a rumen is anaerobic, most of these microbial species are obligated or facultative anaerobic that can decompose complex plant material, such as cellulose, hemicellulose, stench and proteins (Weimer, 1992).

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* Corresponding author: Odeyemi, A. T., Department of Science Laboratory Technology, Ekiti State University, Ado-Ekiti, Nigeria.
E-mail: adebowaleodeyemi@gmail.com

Rumen microbes are of great importance for livestock as they are able to utilize plant nutrients efficiently as source of energy (Das and Qin, 2012). Ruminants have developed a unique microbial symbiosis in the rumen to utilize plant tissue that form a major part of their diet (Quiroz-Castaeda and Folch-Mallol, 2011; and McSweeney and Mackie, 2012).

Rumen has a dense population of bacteria with numbers ranging from 10^8-10^{11} per gram of contents. The number is reflective of the digestibility of the feed, which is why bacterial counts in grain-based diets are 10- to 100-fold higher than the forage-based diets. Majority of the bacteria is obligating anaerobic; although facultative bacteria do exist. Anaerobic bacterial counts are typically about 1000-fold higher than facultative bacterial counts. Most of the facultative bacteria in the rumen are non-indigenous and transient population carried into the rumen through feed and water. Ruminal bacteria are predominantly gram-negative accounting for 80-90% of the population (Castillo-Gonzalez *et al.*, 2014).

Interestingly, it is believed that only 10-20% of rumen microorganisms are known so far; the remaining is still unknown in terms of their identity as well as functions (Kobayashi, 2006). However, the recent applications of cultivation-independent techniques, particularly based on 16S rRNA gene sequence analyses, have indicated that the number of bacterial species in the rumen is vastly underestimated. It is generally believed that culture-based procedures have only identified about 10% of bacterial species present in the rumen (Saro *et al.*, 2012).

The major components of ruminant diets are polymers and include carbohydrates, nitrogenous (protein and non-protein) substances, lipids, and lignins. The polymers, except for lignins, are hydrolyzed to monomers, which are then metabolized to various fermentation products, chiefly acids and gases, depending on the microbial species. The extent to which the polymers are degraded in the rumen depends on the feedstuff and length of retention in the rumen (Bello and Escobar, 1997).

This research aims at characterizing bacterial species and population of the rumen content over 12 days of fermentation and checking out their ability to improve the mineral contents of the rumen substrates for possible soil conditioning and increase of soil fertility for crop high yield.

2. Materials and methods

2.1. Source and collection of rumen substrate

Rumen content of goat was obtained from the slaughter house at Sabo market, Atinkankan, Ado-Ekiti. Samples were aseptically collected and transported in a sterile polythene bag kept in a cooler packed with ice block, to the laboratory of Microbiology Department, Ekiti State University and analyzed within 2 h of sample collection.

2.2. Preparation of rumen substrates and microbiological analysis

Appreciable amount of rumen substrate was thoroughly homogenized and five grammes was distributed into sterile boiling tubes and fermented aerobically and anaerobically over a period of 12 days. Fresh rumen substrate was analyzed immediately as a control experiment. The bacterial load of the samples was determined by the method of Fawole and Oso (2004). Serial dilutions of the various samples were carried out each day of fermentation according to Fawole and Oso (2004). An aliquot (0.1 ml) from the 10^{-8} and 10^{-9} dilution tubes was inoculated using pour plating technique, incubated at 35 °C overnight to enumerate bacteria. Colonies were counted and the microbial load was determined and expressed as CFU/g. The representative colonies were subculture on freshly prepared nutrient agar by streaking to obtain pure strain of bacterial isolates.

2.3. Molecular characterization

DNA was extracted using the protocol stated by (Cho *et al.*, 2006). Polymerase Chain Reaction (PCR) was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA). Agarose gel was ran to confirm amplification. The amplified fragments were sequenced using a Genetic Analyzer 3130 × I sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit (Das and Qin, 2012). BioEdit software and MEGA 6 were used for all genetic analysis (Tamura *et al.*, 2007).

2.4. Molecular analysis of ESBL-coding genes

Molecular investigations of antibiotic resistance gene in our isolates were by simple PCR on the extracted DNA using specific primers to recognize certain antibiotic resistance regions. Primer sequences used were as earlier documented (Mammeri *et al.*, 2005; and Lundberg *et al.*, 2013). Reaction cocktail used for all PCR per primer set included (Reagent Volume μl) - 5X PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10 pM DNTP (0.25), 10

pM of each forward and backwards primer (0.25), 8000 U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 μl template was added. Buffer control was also added to eliminate any probability of false amplification. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

2.5. Mineral analysis of rumen content

The mineral composition of the oven dried samples was analyzed for potassium and sodium using flame photometric method (FP 902 PG) (Skoog et al., 2007). Calcium, magnesium, nitrate and iron were determined using Atomic Absorption spectrophotometer (AAS) Buck Scientific 210 VG (Hoft et al., 1979). While phosphorus content was quantified by ultraviolet-visible spectrophotometry (Cherney, 2000).

2.6. Determination of moisture content

Representative samples, each of which weighed 5 kg, were taken in certain amounts in porcelain cups and dried in an oven at 170 °C for 2 h. Dried samples were kept in a desiccator to be brought down to room temperature (Guyenc, 2016). Samples were weighed again and MC was calculated using equation:

$$M = [(w - d)/w] \times 100$$

where

> w Initial weight of sample, (kg)
>
> d Weight after being dried under 170 °C, (kg)
>
> M Moisture content.

3. Results

A batch culture growth was observed in the bacterial and fungal composition (CFU/ml) of rumen samples of goat for both aerobic and anaerobic fermentation. There was rapid population increase noticed which peaked at the 4th day before a steady microbial culture population till the 8th day followed by a gradual decline observed afterward still day 12 (Tables 1 and 2).

Table 1: Aerobic microbial counts (CFU/ml) of rumen substrate				
Days of fermentation	**Total bacterial count**		**Total fungi count**	
	10^{-8}	10^{-9}	10^{-8}	10^{-9}
1	50	47	25	22
2	49	50	13	10
3	45	30	20	25
4	48	39	20	26
5	70	57	35	30
6	89	60	25	20
7	92	69	30	18
8	80	75	38	25
9	83	79	40	26
10	80	69	25	18
11	55	65	15	13
12	49	62	10	7

Days of fermentation	Total bacterial count		Total fungi count	
	10^{-8}	10^{-9}	10^{-8}	10^{-9}
0	50	47	25	22
1	51	45	19	15
2	59	40	38	30
3	80	77	52	38
4	128	103	59	42
5	128	109	47	29
6	150	109	49	22
7	195	150	67	65
8	100	103	75	72
9	92	100	77	71
10	81	98	45	30
11	75	82	29	18
12	63	79	20	15

Table 2: Anaerobic microbial counts (CFU/ml) of rumen substrate

Twenty-five (25) bacterial isolates were recovered from the rumen samples (13 aerobic and 12 anaerobic). Table 3 showed the aerobic organisms distribution of the rumen content during fermentation. *Bacillus* spp. had the highest frequency of 30.7%, *Pseudomonas* spp. (23.0%), *Escherichia coli* 15.3% while *Proteus* spp, *Shigella* spp, *Lactobacillus* spp. and *Staphylococcus* spp. had 7.6% each respectively. Also, the percentage distribution of isolated facultative anaerobic bacteria revealed *Pseudomonas* spp. having highest frequency of 33%, followed by *Bacillus* spp. and *Lactobacillus* spp. with 25% distribution, *Serratia* spp and *Escherichia coli* with 8.3% each (Table 4).

Table 3: Percentage distribution of aerobic bacterial isolates

Organisms	Number	Frequency (%)
E.coli	2	15.3
Proteus	1	7.6
Pseudomonas spp.	4	30.7
Lactobacillus spp.	1	7.6
Shigella spp	1	7.6
Bacillus spp.	3	23.7
Staphylococcus spp.	1	7.6
Total	**13**	**100**

Table 4: Percentage distribution of facultative anaerobic bacterial isolates

Organisms	Number	Frequency (%)
Serratia spp.	1	8.3
Bacillus spp.	3	25
E.coli	1	8.3
Lactobacillus spp.	3	25
Pseudomonas spp.	4	33.3
Total	**12**	**100**

The selected multiple antibiotics resistant bacteria, *Bacillus anthracis* and *Pseudomonas aeruginosa* were screened for DNA associated with the resistance pattern of the antibiotics to which they were resistant to. They were found to possess different genes for different antibiotic type which indicated that the resistant genes were encoded in the isolated DNA. The isolates possess aadA gene which confirms resistance to streptomycin, with positive amplification of approximately 447 bp indicates gene presence of *Bacillus anthracis* and *Pseudomonas aeruginosa* are molecularly resistant to streptomycin (Figure 1). The isolates possess aac(3)-IV gene which confirms resistance to gentamycin, with positive amplification of approximately 286 bp indicates gene presence of *Bacillus anthracis* and *Pseudomonas aeruginosa* are molecularly resistant to gentamycin (Figure 2). Also the isolates possess catA1 gene which confirms resistance to chloramphenicol with positive amplification of approximately 540 bp indicates gene presence of *Bacillus anthracis* and *Pseudomonas aeruginosa* which are molecularly resistant to chloramphenicol (Figure 3). Finally, the isolates possess aadA gene which confirms resistance to sparfloxacin, with positive amplification of approximately 670 bp indicating gene presence of *Bacillus anthracis* and *Pseudomonas aeruginosa* to sparfloxacin (Figure 4).

The nucleotide sequence analyzed using BLAST bioinformatics suite on the NCBI of a selected isolates revealed their real identities to be *Bacillus anthracis* strain CGS-1 16S ribosomal RNA gene, partial sequence and nucleotide sequence of *Pseudomonas aeruginosa* N17.35 16S ribosomal RNA (Charts 1 and 2).
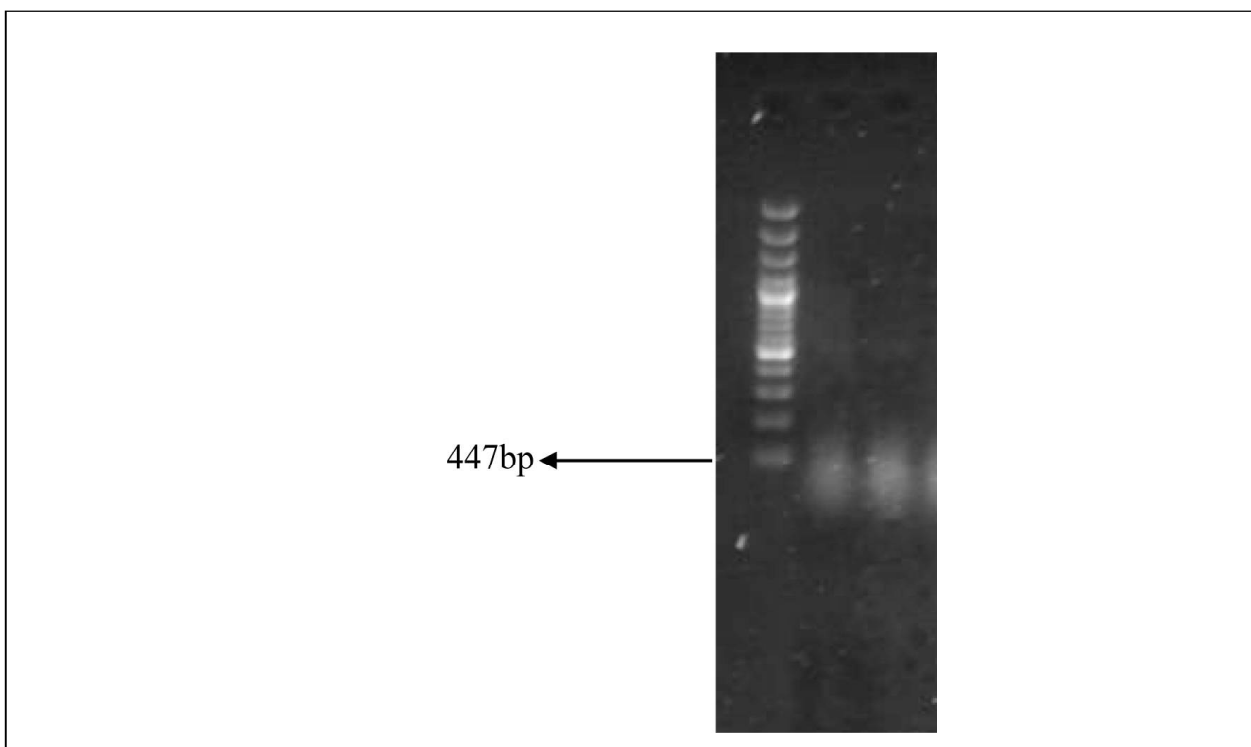

447bp

Figure 1: Isolates possessing aadA gene confirming resistance to streptomycin
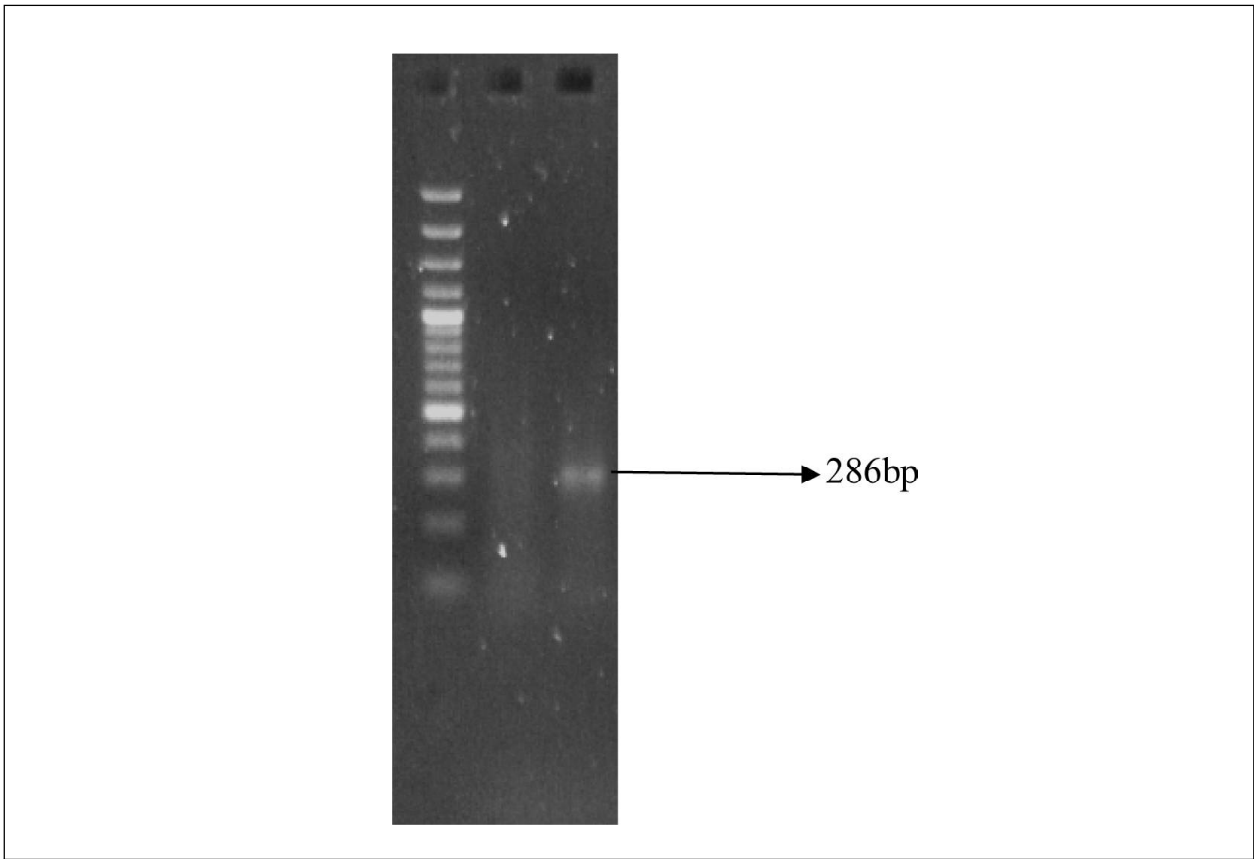
Figure 2: Isolates possessing aac(3)-IV gene confirming resistance to gentamycin
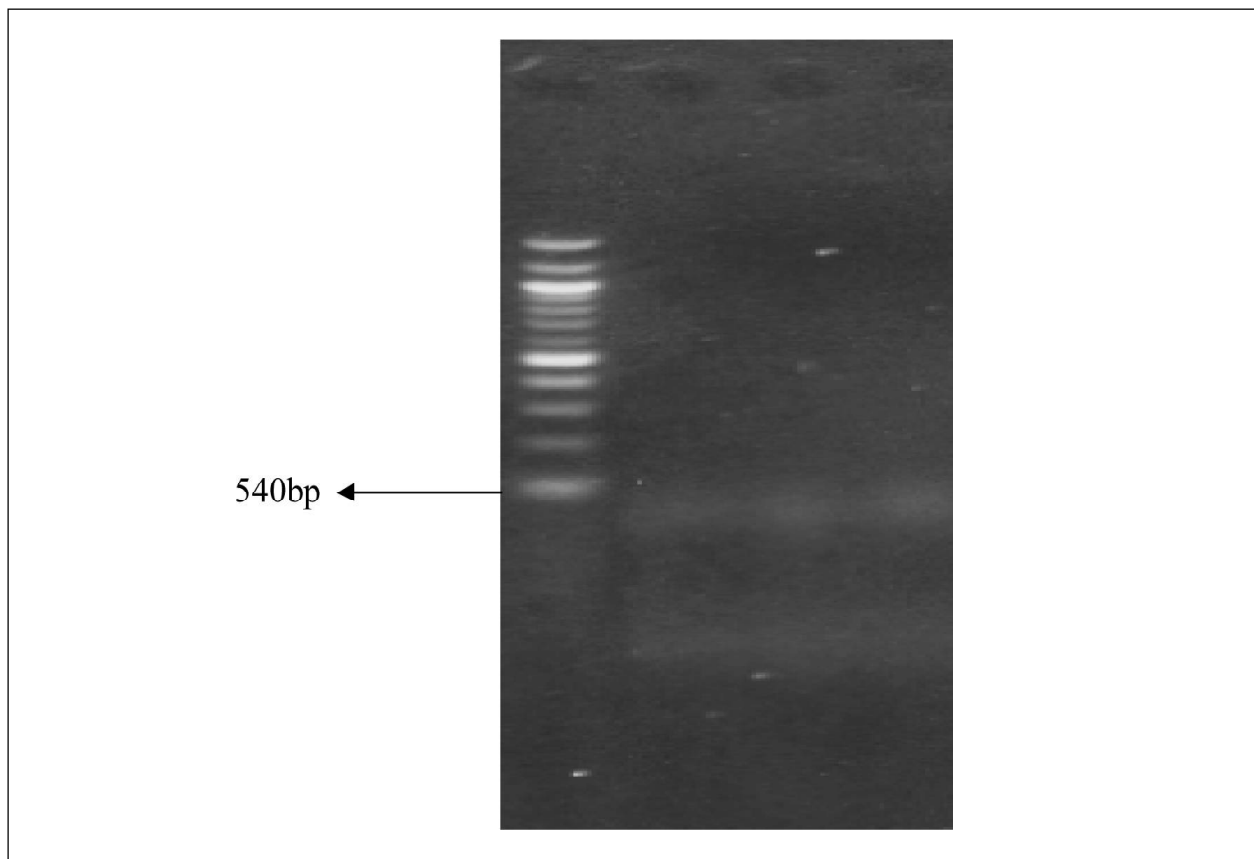

Figure 3: Isolates possessing catA1 gene confirming resistance to chloramphenicol

Figure 4: Isolates possessing aadA gene confirming resistance to sparfloxacin

ACAAATGGAAAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGG
GTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACAT
TTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCG
CGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACC
TGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGGGCAACGCCGCGTGAGTGATG
AAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCT
GGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAG
ACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATA
TGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGC
TAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTG
GGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTG
ACAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGT
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGGCAACCCTTGATCTTA
GTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGAAGGTG
GGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGAC
GGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTC
GGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGC
ATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTT
GTACACCCGGGACT

Chart 1: *Bacillus anthracis* strain CGS-1 16S ribosomal RNA gene, partial sequence

CATGCAAGCCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACNGTTGAGT
AATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGTGAAACGGGCGCTAATACC
GCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCT
AGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGT
CTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCA
GCANTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAA
GAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCT
TGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGT
AATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
CAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGA
GCTAGAGTACGGTAGAAGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATA
GGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGA
AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCG
ACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCT
GGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTAGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGA
GAACTTCCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGT
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGAACCCTTGTCCTTA
GTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGG
TGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATG
GTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAG
TCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCA
GAATGTCACGGTGAATACGTTCCCGGGCCTTGTATCACACCGCCCGTCACACCATGGGAG
TGGGTTGCTCCAGAAGTAGCTAGTCTAACCGCCAAGGGGGATCGGTTATCCACGGAGTG
ATTCATGACCGGGGCGAAGTCGTAACAAGGTAGCCGTAGGGGACTGCGGCTGCAT

Chart 2: *Pseudomonas aeruginosa* **N17.35 16S ribosomal RNA gene, partial sequence**

Table 5:   Mineral estimation (ppm) of aerobic fermented substrates

Days	Na	K	Mg	Ca	P	Moisture content
0	22.70	36.80	1.730	3.880	15.567	3.7
1	24.20	46.00	10.105	11.234	12.125	5.5
2	23.10	44.00	8.178	12.443	13.436	3.9
3	20.00	43.00	7.165	12.235	16.216	3.4
4	22.30	42.00	6.147	14.546	18.456	3.7
5	21.60	42.00	5.135	20.100	20.890	3.6
6	23.40	40.00	5.547	12.745	22.576	3.7
7	26.70	39.00	4.654	17.642	25.673	4.0
8	27.00	38.00	2.550	3.830	8.100	5.7
9	26.00	36.00	4.573	19.367	27.786	3.8
10	23.50	35.20	5.321	21.500	29.200	3.6
11	22.30	34.00	5.543	25.356	29.340	3.0
12	23.70	43.00	4.453	17.200	31.573	2.8

Table 6:	Mineral estimation (ppm) of anaerobic fermented substrates					
Days	Na	K	Mg	Ca	P	Moisture content
0	22.70	36.80	1.730	3.880	15.567	3.7
1	23.90	47.00	1.520	4.100	10.230	6.0
2	21.50	45.00	4.716	16.400	17.590	4.2
3	25.60	35.00	1.576	2.400	5.846	3.4
4	24.00	39.10	5.320	26.200	23.580	3.0
5	20.00	37.10	3.320	17.200	24.580	3.8
6	18.20	33.50	0.597	1.300	28.740	2.7
7	16.50	32.00	4.758	24.300	30.321	5.5
8	15.30	30.00	6.462	23.456	34.675	5.2
9	16.50	32.00	4.758	24.300	37.657	4.6
10	20.00	37.10	3.320	17.200	38.580	5.2
11	24.00	39.00	5.453	26.200	29.573	5.2
12	23.70	43.00	4.453	12.500	40.765	4.5

Table 5 showed the proximate estimation of aerobic fermentation in which different elements such as; sodium (Na), potassium (K), magnesium (Mg), calcium (Ca) and moisture content of which potassium and sodium has the highest value compare to magnesium and calcium. Table 6 below revealed the proximate estimation of anaerobic fermentation in which different elements such as; sodium (Na), potassium (K), magnesium (Mg), calcium (Ca) and moisture content of which potassium and sodium has the highest value compare to magnesium and calcium.

4. Discussion

The rumen is an especially important chamber of the stomach, because it is there that large molecular food is broken down into simpler and utilizable molecules be used by the body. It is full of normal flora microbes that appear as soon as 38 h after the birth of ruminant animal (McAllister *et al.*, 1994). All three major domains inhabit the rumen, but it is thought that bacteria are the most abundant, with 10^{11} viable cells/ml (Yeoman *et al.*, 2011). Without the microbes, the ruminants would get about 15% less nutrients from their food. The ruminalbacteria themselves are also digested by the animals in their small intestines, and further serve as a source of nutrients (Leschine, 1995). There are hundreds of different kinds of bacteria in the rumen which aid in this process, 85-95% which have yet to be cultured and 70% which have not yet been identified (McAllister *et al.*, 1994). *Ruminococcus albus*, and *Ruminococcus flavefaciens* are the principle cellulose degrading organisms (Leschine, 1995). Despite their important role, cellulolytic bacteria are thought to only comprise 0.3% of the total ruminal bacteria population (Yeoman *et al.*, 2011).

In this study, the bacterial load of both aerobic and anaerobic fermentative cultures exceeds fungal load. The distinguishing variation in microbial estimation obtained in this work agrees with the result obtained by Kamra (2005) in which the efficiency of ruminants to utilize a wide variety of feeds (Leng, 1990) was based on a highly diversified rumen microbial ecosystem consisting majorly of bacteria (10^{-8}, 10^{-9} cells/ml). Many of bacteria in the rumen are free-floating in the liquid just before feeding and become attached to new feed particles after feeding. It is very difficult to remove and count the bacteria attached to feed particles and thus may explain the low numbers observed in the rumen after feeding, when fermentation rate is generally at its greatest. Bacterial numbers are generally assumed to be higher on high concentrate diets compared to high forage diets (Hespell *et al.*, 1997; and Morgavi *et al.*, 2010). However, there are more fluid-associated bacteria with high concentrate diets and thus, easier to enumerate. The biggest differences due to diet are in the type of bacteria rather than the total number.

A preliminary microbiological analysis was carried out to know which organisms are consistent and adapt under both anaerobic and aerobic conditions in improving the mineral content of the substrate, during which selected bacteria (*Bacillus anthracis* and *Pseudomonas aeruginosa*) at high value of mineral content indicate the beneficial activities of these species of bacteria in utilizing rumen substrates as a possible source of biofertilizer. The most effect of microbial feed additives is that increasing the viable count of anaerobic bacteria recovered from ruminal fluid by 50-100% (Wallace and Newbold, 1993). Effects of microbial preparation and substrate have been fairly well-documented, but little information on interaction between microbial fraction and substrate is available in the literature. Forages account for 30% to 100% of ruminant rations, which has been reported to influence the microbial community composition differently both in-vivo and in-vitro (Kong *et al.*, 2010).

Antibiotics resistance test was performed to know whether the normal flora bacteria isolated could be causative agent of infections by possessing resistant gene for the transfer of infection amongst the isolated strains. When those microbes develop resistance towards some antibiotics, it is highly needed to treat the rumen substrates for the production of biofertilizer, preventing crops from being infected and eventually infecting those crop consumers.

Most of the resistance displayed by the isolated strains in this research might be from the water dosed with varied antibiotics to cure infections of the livestock as reported by Doyle *et al.* (2013). Hence, indiscriminate treatment of livestock with antibiotics should be disallowed because of the secondary effect on the animal and the indirect influence through plants to humans.

Under conditions similar to those of our study, Arthington and Swenson (2004) found differences between seasons in contents of Ca, P, Na and Mg. According to McDowell (2003) and Haenlein and Ramirez (2007) these differences in the mineral content of diets may be attributed to the interaction of a number of factors including soil, plant species, yield, pasture management, climate (temperature and rainfall) and stages of maturity.

5. Conclusion

Rumen content of goats is low cost manure that can be used for biofertilizer production, to help in fast growth of plant due to its high nutritional value and available fermentative/utilizing microbes. These microbes most especially the molecular identified bacteria have the tendency to ferment the rumen substrate which can be further processed and utilized for the production of biofertilizer. The major nutrient composition of rumen substrates from goat slaughtered around Atinkankan in Ado-Ekiti, pose a view to exploiting its alternative as a potential biofertilizer. Further research on rumen substrate is highly needed for the extension of biofertilizer usage to a large number of farmers for improvement of crop yield and other benefits, while minimizing environmental pollution from agrochemical inputs.

Also further studies on the use of rumen content of goat in production of biofertilizer and comparison of effectiveness with chemical or plant fertilizers is ongoing. Meanwhile, antibiotics should not be used indiscriminately on the livestock to prevent aftermath effects on the animal waste product for the production of biofertilizer.

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