

Isolation And Identification Of Bacterial Diversity In Rumen Fluid Of Cow, Goat And Chicken

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Abstract: *Biogas is now an ecologically sound option to reduce environmental burden by decomposing organic material and producing not only energy but also good quality organic fertilizer. Studies on biogas production by co-digestion of animal wastes with grass have attracted special interest today. Poultry waste, cow dung and other waste have been used for biogas production, but the efficiency of the gas production is efficient. From the cow rumen fluids, goat and chicken gut nine different organisms was identified from each sample and they were 16S rRNA sequenced and submitted to NCBI and got the Accession Number.*

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I. Introduction

The utilization of fossil fuels on a global scale is limited by the availability of these resources and by the environmental effects of their excessive exploitation. The production of renewable energy carriers is therefore currently increasing the attention worldwide. Biogas is a regarded as the gifted entrant in which the technology poses its production that may unite the treatment of various organic wastes with the generation of an energy carrier for the most versatile applications. There is a great deal of environmental pressure in many parts of the world to ascertain how a livestock waste. Anaerobic digestion has been considered as waste-to-energy technology that is widely used in the treatment of different organic wastes, For example: organic fraction of municipal solid waste, sewage sludge, food waste, animal manure etc.

Many anaerobic biomethanization studies from digestion of cattle manure and co-digestion of fruit and vegetable wastes, organic household wastes, industrial organic wastes and sewage sludge wastes are reported. Lignocellulosic biomass is a renewable and carbon-neutral resource that can be found abundantly and low in cost. However, the characteristics of the lignocellulosic materials itself became the major barrier for the efficient conversion of cellulose and hemicelluloses into monosaccharide that can be subsequently fermented into biogas. Therefore, the biomass has to be maintained with proper environmental conditions at proper temperature particularly in the absence of oxygen for effective formation synergistic relationship between microbial communities.

Biogas is an environmental friendly and one of the most efficient and effective options for renewable energy among various other alternative sources. Biogas is a mixture of flammable gas produced through anaerobic digestion process of organic materials. It is mainly composed of CH₄ (45-70%), CO₂ (30-45%), traces of H₂, water vapour (H₂O), ammonia (NH₃) and hydrogen sulphide (H₂S). It is chiefly used to fuel applications, from cooking stove to generating electricity.

II. Materials And Methods

The cow rumen fluid, goat gut and chicken gut samples were collected from the Namakkal Veterinary College. The collected gut fluid samples from cow, chicken and goat was further processed and analyzed for microbiological analysis. The samples were serially diluted upto 10⁻⁹ dilution. The plates were incubated for 24 hours and observed the bacterial growth.

DNA Sequencing was carried out using a Universal sequencing primer. All sequences were compared with similar sequences of the reference organisms by BLAST search. The sequences were submitted to NCBI.

III. Results

Colony counts of bacterial species from rumen fluids of different animals showed highest bacterial colony counts in cow rumen fluid (434.33) followed by goat (262.67) and chicken (170.67). Nine bacterial species were isolated from cow rumen fluid, goat and chicken and they were 16s RNA sequenced and submitted to NCBI and got the Accession Number are listed in the table1.

S. No.	Cow rumen	Goat	Chicken
1.	Acetobacterium woodii Accession Number: MH709096	Acetobacterium woodii Accession Number: MH707021	Acetobacterium woodii Accession Number: MH708234
2.	Eubacterium ruminantium Accession Number: MH709097	Clostridium carboxidivorans Accession Number: MH707022	Clostridium cellobioparum Accession Number: MH708235
3.	Methanobacterium bryanti Accession Number: MH709098	Lactobacillus acidophilus Accession Number: MH707023	Ruminococcus flavifaciens Accession Number: MH712066
4.	Clostridium carboxidivorans Accession Number: MH704600	Methanobrevibacter ruminantium Accession Number: MH707024	Eubacterium xylanophilum Accession Number: MH708236
5.	Fibrobacter succinogenes Accession Number: MH712044	Ruminobacter amylophilus Accession Number: MH707025	Methanobrevibacter smithii Accession Number: MH708237
6.	Methanobrevibacter ruminantium Accession Number: MH709100	Succinimonas amylolytica Accession Number: MH707026	Methanosarcina thermophila Accession Number: MH708238
7.	Prevotella ruminicola Accession Number: MH709101	Ruminococcus albus Accession Number: MH707027	Prevotella ruminicola Accession Number: MH708239
8.	Ruminobacter amylophilus Accession Number: MH709102	Selenomonas ruminantium Accession Number: MH707028	Ruminobacter amylophilus Accession Number: MH708240
9.	Selenomonas ruminantium Accession Number: MH709103	Streptococcus bovis Accession Number: MH707029	Streptococcus bovis Accession Number: MH708241

Colony counts of hydrolyzing bacterial species from rumen fluids of different animals showed highest colony counts in cow rumen fluid (549.33) followed by goat (444) and chicken (316). Colony counts of acidogenic bacterial species from rumen fluids of different animals showed highest colony counts in cow rumen fluid (268) followed by goat (208) and chicken (146.67). Colony counts of methanogenic bacterial species from rumen fluids of different animals showed highest colony counts in cow rumen fluid (88) followed by goat (54.67) and chicken (32).

IV. Discussion

The microorganisms in the rumen region of animal have symbiotic relationship with the animal and also the microbes in the gut region of chicken where the host animal provide biophysical conditions and nutrient to the microbes in turn the microorganism provide energy, vitamins, proteins from the food they ingest to the host. Microbial populations of the animal rumen and gut region harbor all three domains Eukarya which include protozoa and fungi, Eubacteria which include bacteria and archaea which include methanogens.

In many cases bacteria of cellulolytic activity also possess pectinolytic, proteolytic and amylolytic activity which are important in breaking down complex protein into smaller polypeptides and amino acids, bacteria involved in these activities are dominant in the rumen fluid and gut fluids such as Ruminobacter amylophilus, Selenomonas ruminantium, Succinimonas amylolytica, Streptococcus bovis, Lactobacillus sp. etc. Prevotella ruminicola identified in the present study from cow rumen and chicken gut fluid is a proteolytic bacterium predominant in the rumen fluid (Griswold and Mackie, 1997).

Acetobacterium woodii was identified in all the three animal samples cow rumen, goat rumen and chicken gut which are known for their methane mitigation through their acetogenic activity which is also reported by Joblin, (1999). Methanogenic are archaeal bacterial species identified separately from bacterial protozoa and fungi represents 2 to 4% of the rumen bacterial population lack in peptide glycan with unique lipid structure (Nagaraja, 2016). In the present study three genera and five species have been identified Methanobacterium bryantii from cow rumen which was found to be one of the efficient methanogenic bacteria which has large pH gradient (Jarrell and Sprott, 1983; Sprott and Jarrell 1984). Methanobrevibacter ruminantium identified in rumen fluid of cow and goat and M. smithii from chicken gut reduce carbon dioxide using hydrogen and form methane (Dighe et al., 2004). Methanosarcina thermophila is a thermophilic bacteria identified from chicken gut in the present study reduces acetate, methanol and amines into methane (Clements and Ferry, 1992). However, in the present study rumen and gut fluids are dominant with hydrolytic bacteria followed by acidogenic, acetogenic and methanogenic bacteria in the sequence of cow, goat and chicken gut.

V. Conclusion

The rumen is a complex ecosystem that possesses a large variety of microorganisms, that includes bacteria, protozoa, archaea and fungi. The major function of the microbiome present in the rumen is the

conversion of plant materials into digestible compounds that can be used by the animal host. Furthermore, as the microbes in the rumen undergoes long-term selection and evolution, the microbes and host form an inter inhibitive and interdependent homeostatic relationship that has an important role in maintaining host health, improving performance, reducing environmental pollution, and ensuring food and animal product safety. Clostridia have been shown to be the most common bacteria degrading cellulose and anaerobic digesters, while *Flavobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavifaciens* are dominant cellulose degraders in the rumen improves anaerobic digestion in artificial systems.

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