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Research article

ISOLATION OF METHANOGENS FROM TERMITE GUT AND ITS ROLE IN BIOGAS PRODUCTION BY USING POULTRY WASTE

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ABSTRACT: To achieve a functioning and stable process with high methane production it is important to create and maintain a beneficial environment for the activity of bacterial consortia of suitable species. Therefore, the present study was carried out to isolate methanogen from termite gut and analyze its role in biogas production. The biogas production was carried out in a pilot scale batch reactor for 30days using poultry waste as a substrate. Morphological, biochemical characterization and molecular identification has been done tothe isolated methanogen. The growth of isolated methanogen was tested under different pH, temperature and different substrates. The physico-chemical parameters such as pH, temperature, Chemical oxygen demand and total solids were measured in the interval of 10 days. Biogas production was analyzed between the control and treated biogas tanks in order to identify the effectiveness of biogas production.
Key words: Methanogen, Termite gut, biogas, Poultry waste.

INTRODUCTION

The demand of the world economy for electrical and thermal energy in over 88% is covered from non renewable energy resources mainly petroleum and natural gas [8]. The rising cost of petroleum products is a serious problem facing most developing countries of the world including Nigeria. Again, excessive energy demands from both rural and urban dwellers imply that other natural sources of energy have to be explored. Hence, conversion of agricultural wastes into biogas could be a leeway to solving some of these energy problems. Biogas production is a complex biochemical process that takes place in the absence of oxygen and the presence of highly sensitive microorganisms that are mainly bacteria [9]. The flammable biogas consists of CH_4, CO_2 with traces of gases like H_2S, NH_3, CO, H_2, N_2 and water vapour etc. [5]. Methane fermentation is a complex process, which can be divided up into four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Methanogenesisor biomethanation is the formation of methane by microbes known as methanogens. Organisms capable of producing methane have been identified only from the domine Archaea, a group phylogenetically distinct from both eukaryotes and bacteria, although many live in close association with anaerobic bacteria. Methanogens are strict anaerobes which share a complex biochemistry for methane synthesis as part of their energy metabolism. The production of methane is an important and widespread form of microbial metabolism. In most environments, it is the final step in the decomposition of biomass. Methanogens are observed in anoxic underground environments, contributing to the degradation of organic matter.In search of effective methanogens, animal gut has been found to be important spot to identify. Methanogenesis occurs in the guts of humans, termite and other animals. Methane is a minor component of microbial carbon metabolism in the hind gut of termite digestive system. The present study was aim to isolate methanogens from termite gut and study its role in biogas production.

MATERIALS AND METHODS

Sample collection and isolation of methanogenic bacteria from termite gut

The termites were collected in a clean plastic container from local area of Salem. The termites taken out of their nests are placed in sterilized Petri plates for surface sterilization by 70% alcohol. Gut sample were removed from stomach region of the termite under dissection microscope using sterile needle and it was serially diluted up to 10^{-8}. Then it was pour plated on methanogenic enrichment medium. The inoculated plates were incubated in an anaerobic chamber for 3-5 days.

Morphological and biochemical parameters

Morphological and biochemical characteristics of methanogenic bacteria was determined using gram staining, motility,IMViC, catalase, oxidase, triple sugar ion, glucose, sucrose and lactose.

16sr RNA Sequencing

The DNA was isolated from the pure culture and it was amplified using M23FB and M1382R primers in PCR. The PCR was performed by denaturation at 94^0C for 30 sec, 52^0C for 45 sec, and 72^0C for 2 min and final elongation step of 10 min at 72^0C. The PCR product was purified and used for direct sequencing. Sequencing primer used were

M520R-(5'–TACCGCGGCGGCTGGC-3'),
800F-(5'-ATTAGATACCCCGGGTAG-3').
Ar1000R-(5'-TCTCCGCTCGTTGCCTGACT-3') and
Ar1000F-(5'-AGTCAGGCAACGAGCGAGA-3').

DNA sequencing was performed with a 3700 automatic sequence analyser. The sequence data obtained from 16S rRNA submitted in NCBI and identified through BLAST. Sequences were aligned using the clustalX package version then checked manually.

Growth Kinetics

Growth kinetics of methanogen was studied under different temperature (15, 30, 45, 75, and 90^0C) and pH of (4, 5, 6, 7, 8, 9, and 10). The growth of the organism was also studied using different concentration (0.04, 0.08, 0.12, 0.16, 0.2, 0.24, 0.26 gm/ml) of sucrose, fructose and lactose. The growth rate was measured by spectrophotometer at 660nm.

Anaerobic Digestion

Sample collection and experimental setup

10 days old poultry waste was collected from in and around Namakkal. The poultry waste was blended with water in the ratio of 1:5 and loaded into self designedbiogas tank. The capacity of the biogas tankwas17 L. Pure broth culture of methanogenabout 1.5L was added into one biogas tank and it was named as treated. Without any inoculum the biogas tank was named as control.

Analytical methods

The physico-chemical parameters such as temperature, pH, Total solids (TS) and Chemical Oxygen Demand (COD) were performed according to the standard methods by APHA(1998) [2].

Microbial analysis

Total viable count for the poultry waste was carried out to determine the microbial load of the sample. This was carried out in two different periodsie. before and end of the anaerobic digestion. Total viable count was calculated by using this formula.

No. of bacteria/ml = No. of colonies/dilution x amount plated

GC Analysis

The gas was collected from the biogas tanks on 10^{th}, $20^{th}$and 30^{th} day.The gas produced in the biogas tanks were analyzed by using GC3800 gas chromatograph fitted with a flame ionization detector (FID); a glass column (1.6m long, 3mm i.d.) packed with 80/100 porpak Q; flow rate (N_2) 25ml/min; column temperature 32^0C; detector temperature 50^0C.

RESULTS

Isolation, morphological and biochemical characteristics of isolated methanogen

The methanogenic organism was isolated from 10^{-5} dilutionafter 4 days of incubation. Then it was pure cultured and maintained onmethanogenic enrichment medium. The morphology of the methanogen showed purple colony, gram positive coccus and non motile. The biochemical characteristics of the isolated methanogen showed positive to methyl red, vogusproskauer, citrate, catalase, oxidase, triple sugar iron, glucose, sucrose, lactose test and negative to indole test.

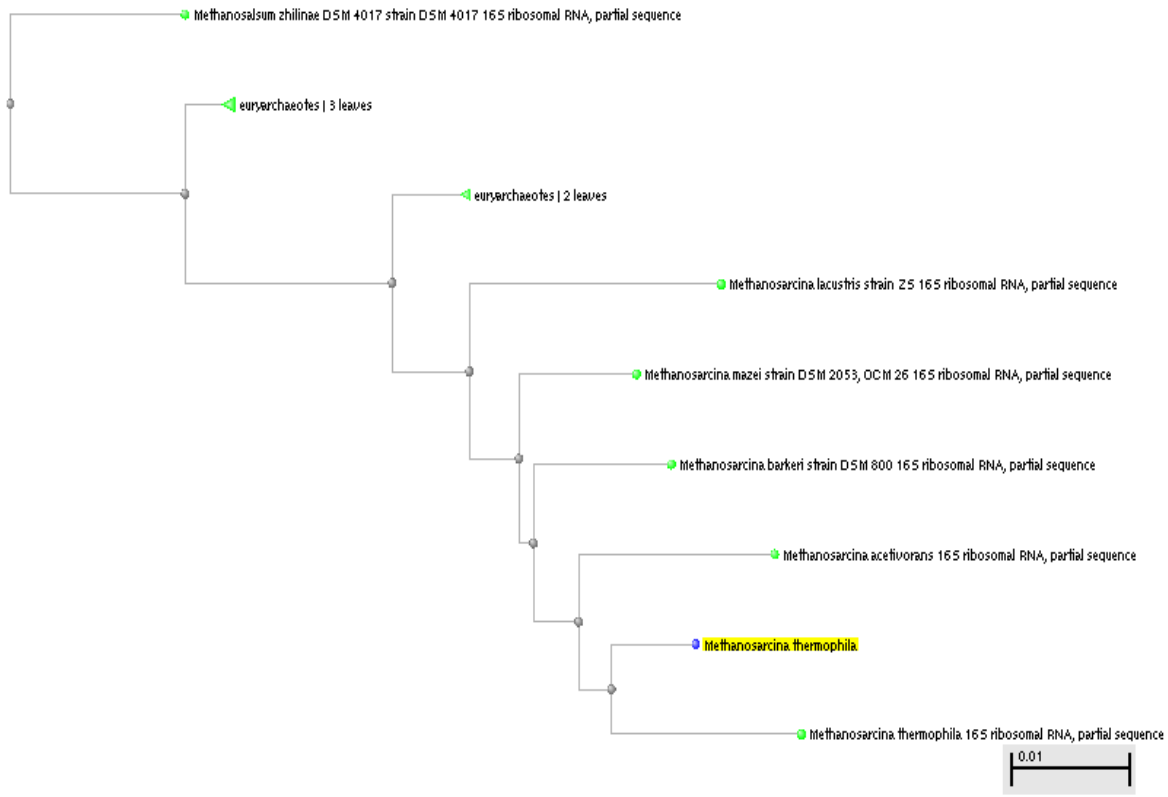
16SrRNA Sequencing

DNA isolated from the pure culture was amplified and the amplicon showed clear band at 1500kb. 16SrRNA sequencing yielded 1261 base pairs with 303 adenine, 313 cytosine, 405 guanine, and 246 thiamine. The BLAST analysis revealed that the isolated methanogen was *methanosarcina thermophile* (box1).

Growth kinetics of Methanogen

Growth kinetic study showed that *Methanosarcina thermophile* was found to be growing well at 75^0C. After 75^0C the growthrate was found to be get reduced (Fig:1). Among different pH tested the organism's growth was found to be high at pH 8.00(Fig: 2). Under different concentration of carbon sources the growth of the *Methanosarcina thermophile* showed higher growth in glucose followed by sucrose and least in lactose (fig.3).

Box 1. Blast tree



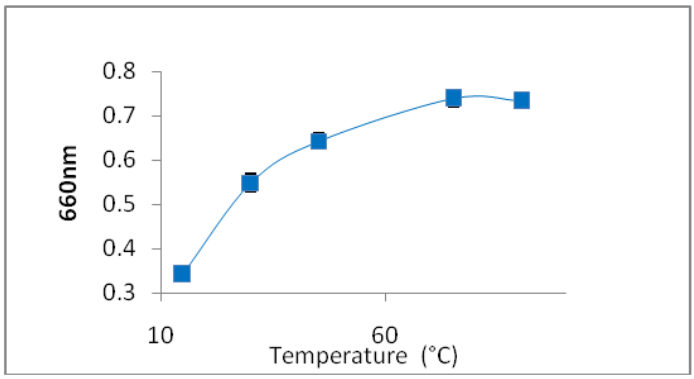


Fig-1: growth of *Methanosarcina thermophila* at different temperature

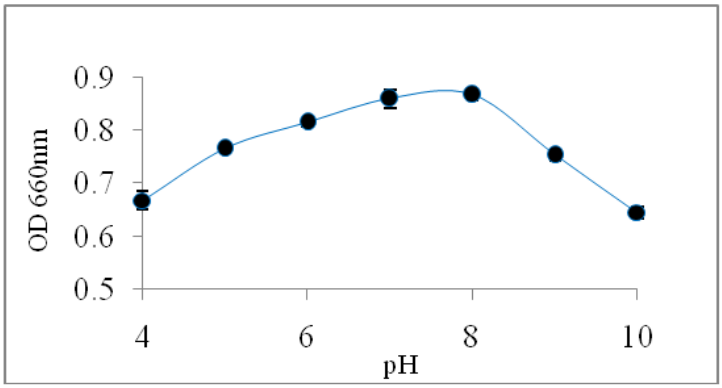


Fig-2: growth of *Methanosarcina thermophila* at different pH

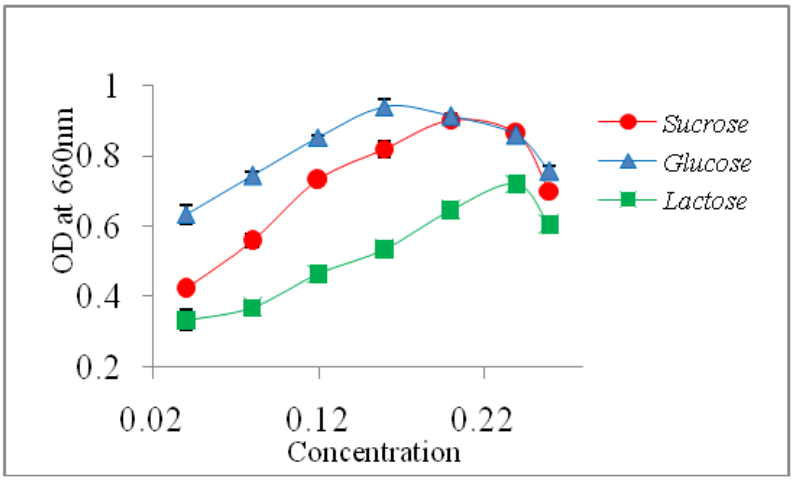


Fig-3: growth of *Methanosarcina thermophila* at different substrates

Physico-chemical parameters and microbial population

pH in the biogas tank ranged from7.7 to 7.9.Here the increasing trend (table 1)in pH was observed. Total solid and COD were decreased at the end of anaerobic digestion (30th day) when compared with the initial day (0th day). The total solid reduction (table 1) ranged from 16.98-15.1 mg/ml and COD reduction (table-1) was ranged between 14.5-11.8mg/ml.Microbial population of poultry waste showed initially1.96×10^{-5} and at end of the digestion it showed 2.4×10^{-5}. Microbial population was increased towards the end of anaerobic digestion.

Table-1: Physico chemical parameters during anaerobic digestion

No	Parameters	0th day	30th day
1	P^{H}	7.7	7.97
2	Total solids	16.98	15.1
3	COD	14.5	11.8

Biogas Production

Biogas production revealed that the CO_2 emission was high at initial stage (0-10th day). This scenario changed after 20th day with increasing release of methane. High concentration of methane 74.29% was obtained at 30th day of anaerobic digestion in treated biogas tank when compared with the control which gave only 48.03% of methane (fig.4).

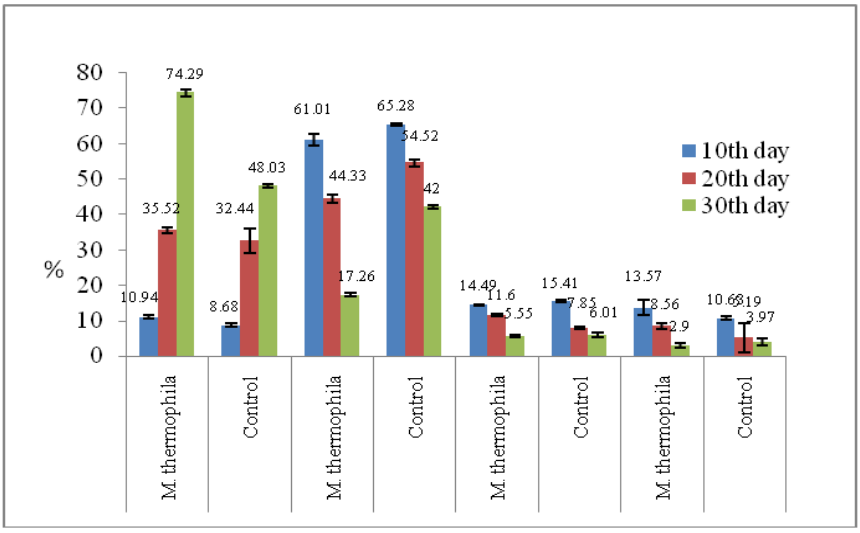


Fig-4: Biogas production in biogas units with poultry as substrate

DISCUSSION

Methane is produced as a metabolic by product in anoxic conditions by methanogenic microorganisms belongs to Archaea [10]. The methanogenic organism *Methanosarcina thermophile* was isolated from termite gut. *Methanosarcina thermophile* is a thermophilic, methane producing bacterium.Methanogens are abundant microorganisms in the termite gut which releases methane also reported as a significant source of global atmospheric methane [9]. Biochemical study of *Methanosarcina thermophile* showed that it was are gram positive, which indicates peptidoglycan in the cell wall with DD-transpeptidase indicates that it act upon the products of proteins from hydrolysis phase and peptides are being degraded to release more propionic acid, CO_2 and hydrogen in the substrate for methanogenesis [13]. Growth kinetics of *Methanosarcina thermophile* was studied under different concentration of carbon sources like glucose, sucrose and lactose showed high growth was found in glucose at the concentration of 0.16gm/ml. Methanogen has an unusual type of metabolism, because they use H2/CO2, formate, methylated C1compounds or acetate as energy and carbon sources for growth [10]. The optimum temperature for growth of *Methanosarcina thermophile* was around 75^0C because it is a thermophilic bacterium. Stephen et al., (1979)[14] isolated a thermophilic strain of Methanosarcinafrom a laboratory-scale 55°C anaerobic sludge digester. He stated that Methanosarcina strain had a temperature optimum for methanogenesis near 50°C and grew at 55°C. The optimum pH for *Methanosarcina thermophile* was around 7-8. Clarens and Moletta (1990) [3] studied the effect of temperature in the thermophilic methanogenic bacterium Methanosarcina sp. MSTA-1. They found the optimum pH for this strain was about 6.5-7.5.

The physico-chemical parameters such as pH, TS, COD were studied during the anaerobic digestion. The anaerobic degradation process is highly pH dependent because each of the microbial groups involved in the reactions has a specific pH range for optimal growth. The aspects influenced by pH include utilization of carbon and energy sources, efficiency of substrate dissimilation, synthesis of proteins and various types of storage material, and the release of metabolic products from the cell [4]. The pH during biogas production was found to be slightly increased when compared with the initial day. This is due to the optimal pH for methane-producing microbes are 6.8-7.2.The growth rate of methanogenic microbes decreases sharply below pH 6.6 [11]. The higher rate of total solids reduction during the study period indicated proper digestion has been takes place because total solid destruction is a vital aspect in evaluating anaerobic digestion performance.The COD removal efficiency of poultry waste during the anaerobic digestion showed decreased when compared with the initial day.Similar result was reported by Abubakar and Ismail(2012)[1]. He stated that the COD removal efficiency during the anaerobic digestion of cow dung was 48.5%, which is comparatively lower than the commonly obtained COD removal from cattle manure (51-79%).This result was believed to be due to high COD exhibited by the inoculum which leads to increase in influent COD concentration. In effect, it dominates the microbial activity thereby resulting in lower COD removal efficiency.

Microbial population during anaerobic digestion showed higher while compared with an initial days. Microbes play crucial role in anaerobic digestion of each and every stage. For enhanced biogas production it is important to understand the microbial community structure and dynamics during the operation time. Higher microbial population leads to higher degradation rate of organic matter and higher biogas production. Similar results were obtained by Ofoefule et al., 2010 [12]. He analyzed the microbial load during the biogas production from paper waste. He stated that the microbial load was lower at starting and increased towards the peak of production.

Biogas production during anaerobic digestion showed that the biogas tank treated with *Methanosarcina thermophile* gave utmost methane yield 74.29% when compared to the control it gave only 48.03%. It clearly showed that the inoculation of methanogenic organism has the ability to enhance the biogas production. Gopinath et al., (2014)[6] reported that the treatment of different microbial consortia, the consortia 4 treated biogas unit gave maximum methane yield 79.45% at 30th day of anaerobic digestion since it contains high amount of methanogenicarchaea.

CONCLUSION

The methanogen isolated from termite gut was *Methanosarcina thermophile* confirmed by 16s rRNA sequencing analysis. From this study it was concluded that *Methanosarcina thermophile* can able to enhance the biogas production.

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