

# Isolation of High Lignolytic Bacteria from Termites's Gut as Potential Booster in for Enhanced Biogas Production

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## Authors' contributions

This work was carried out in collaboration among all authors. Author USA wrote the first draft of the manuscript and managed the analysis of this study. Author JNO designed the study. Author CA performed the statistical analysis of this study and author WB handled the literature searches.

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## ABSTRACT

Bacteria strain capable of degrading lignin, cellulose and hemicellulose were isolated from wood Feeding termite gut using spread plate technique. The 16S rRNA gene sequencing methodology was adopted in the identification of the isolate. The isolate's *Morganella morganii* (strain S4L2C (MH745964) were found to have a high lignin degradation potential. The organism was able to reduce the lignin content of rice straw from 17.43% to 7.29% after 30 days of pretreatment with 53.27% reduction of the lignin content. This study revealed that termite's gut bacteria are Potential sources of lignocellulose degrading bacteria for the biological conversion of biomass to biogas production.

**Keywords:** *Biodelignification; termites gut; gene sequence; biogas.*

## 1. INTRODUCTION

Termites play a great role in terrestrial ecosystem by recycling lignocellulosic biomass,

which refers to a mixture of cellulose, hemicellulose and lignin. Termites are one of the most important soil insects that efficiently decompose lignocelluloses with the aid of their

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associated microbial symbionts. Termites are said to dissimilate a significant proportion of cellulose (74-99%) and hemicellulose (65-87%) components of lignocellulose they ingest. This insect play an important role in the turnover and mineralization of complex biopolymers, such as wood and other cellulose and hemicelluloses containing materials [1].

Lignocellose is the most predominant component of the woody and dead plant materials, as well as the most abundant biomass on earth, especially in terrestrial ecosystems.

In recent years, increasing gas prices and environmental concerns have become the driving force for developing alternative energy sources, especially biogas for automobiles. Currently, Rice straw is the primary raw material for biogas production. However, lignocellulosic biomass has the potential to provide a more economical feedstock as a result of its widespread availability, sustainable production and low starting value.

Existing pretreatment methods have largely been developed on the basis of physicochemical technologies such as steam explosion, dilute acid, alkali, and oxidation or varied combinations [2]. However, typical physical and chemical pretreatments require high-energy (steam or electricity) as well as corrosion-resistant, high pressure reactors, which increase the cost of pretreatment and need for specialty equipment. Furthermore chemical pretreatments can be detrimental to subsequent enzymatic hydrolysis and microbial fermentation apart from producing acidic or alkaline waste water which needs pre-disposal treatment to ensure environmental safety [3]. Microbial pretreatment employs microorganisms and their enzyme systems to breakdown lignin present in lignocellulosic biomass. This environment friendly approach has recently received increased attention and has potential advantages over the prevailing physicochemical pretreatment technologies due to reduced energy and material costs, simplified processes and equipment, and use of biologically based catalysts. The present work mainly focused on isolation of lignolytic bacteria from termites gut, its gene sequencing and the study of its lignolytic efficiency using rice straws a potential booster for biogas production.

## 2. MATERIALS AND METHODS

### 2.1 Sampling Site

The wood feeding termites (*Cryptotermes brevis*) were collected from Nekede community which is

5°26'0" North and 7°2'0" east of Owerri city in Owerri West Local Government Area of Imo State (Give geographic coordinates).

### 2.2 Extraction from Termites

The termites were taken out of their nests and placed in sterilized petriplates. They were then surface sterilized by washing with 70% alcohol. Each termite was separated into its head and body. After removing the heads with forceps, the bodies were crushed with the help of glass rods. The paste obtained from the termites' gut was used for isolation of the bacteria with the help of inoculating loops [4].

### 2.3 Isolation of Bacteria from the Termite Gut

A 10 fold serial dilution was done, where 1g of the squashed termites gut was put in 9mL of maximum recovery diluent (MRD) and left to stand for 30mins – 1 hour, then 0.1 mL was collected from the maximum recovery diluent and dropped on an enriched medium(containing 2% w/v saw dust powder, 0.05% w/v glucose, 5% v/v stock salt solution, 0.02% v/v Hunter's trace element and 1.5% w/v agar)and incubated at 26°C for 60 days.

### 2.4 16S rRNA Gene Sequence of the Isolated Organism

#### 2.4.1 DNA extraction (Boiling method Yamagishi J, et al. 2016)

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 500 µL of normal saline and heated at 95°C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm.

The supernatant containing the DNA was transferred to a 1.5 mL micro centrifuge tube and stored at -20°C for other downstream reactions.

#### 2.4.2 DNA quantification (Wilfinger WW, et al. 2006)

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2µL of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded

onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

#### 2.4.3 16S rRNA amplification (Relman, 1999)

The 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 3'-CGGTTACCTTGTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles.

The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5 µM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.

#### 2.4.4 Sequencing (Lander, et al. 2006)

Sequencing was done using the Big Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10 µl, the components included 0.25 µl BigDye® terminator v1.1/v3.1, 2.25 µL of 5 x BigDye sequencing buffer, 10 µM Primer PCR primer, and 2-10 ng PCR template per 100bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4mins.

#### 2.5 Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0. The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [5].

#### 2.5.1 E. test for the lignolytic efficiency of the isolated bacteria

An inoculum of the isolated bacteria was used to pretreat Rice Straw for a period of 30 days within which the cellulose, hemicellulose and lignin composition of the rice straw was monitored at 15 days interval which was done according to methods described by Lin [6].

- Reflux the sample at 80°C with 0.5 M NaOH for 4h(100 ml/g of biomass) filter and wash the residue with distilled water until neutrality = Hemicellulose.
- Soak another sample at room temperature in Conc. Sulphuric acid (30 ml/g of biomass) for 24h, then reflux at 100°C for 1h, filter the residue and wash with distilled water to neutrality = Lignin.
- Cellulose = lignin – Hemicellulose.

### 3. RESULTS AND DISCUSSION

The obtained 16s rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolate F1 showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolate F1 within the *Morganella* sp. and revealed a closely relatedness to *Morganella morganii* strain S4L2C (MH745964) than other *Morganella* sp. as can be seen in (Fig. 1).

Changes in chemical composition of biopretreated rice straw samples are given in Table 1. Rice straw samples were analyzed for change in the lignin composition at three intervals which is on the day of charging zero day after the 15<sup>th</sup> day and 30<sup>th</sup> day of pretreatment and a significant change was seen in the chemical composition of biotreated samples expect the value got at the 15<sup>th</sup> day which rather increased from that got at the 0 day which could be attributed to either the type of substrate and operational conditions (Speece, 1983). As shown in Table 1, *Morganella morganii* strain S4L2C (MH745964) strain significantly degraded and mineralized lignin to release the trapped cellulose and hemicellulose in the rice straw. Maximum lignin removal of about 53.74% was achieved after the 30<sup>th</sup> day of pretreatment. Lignin degradation was monitored by measuring the lignin content of the rice straw at intervals

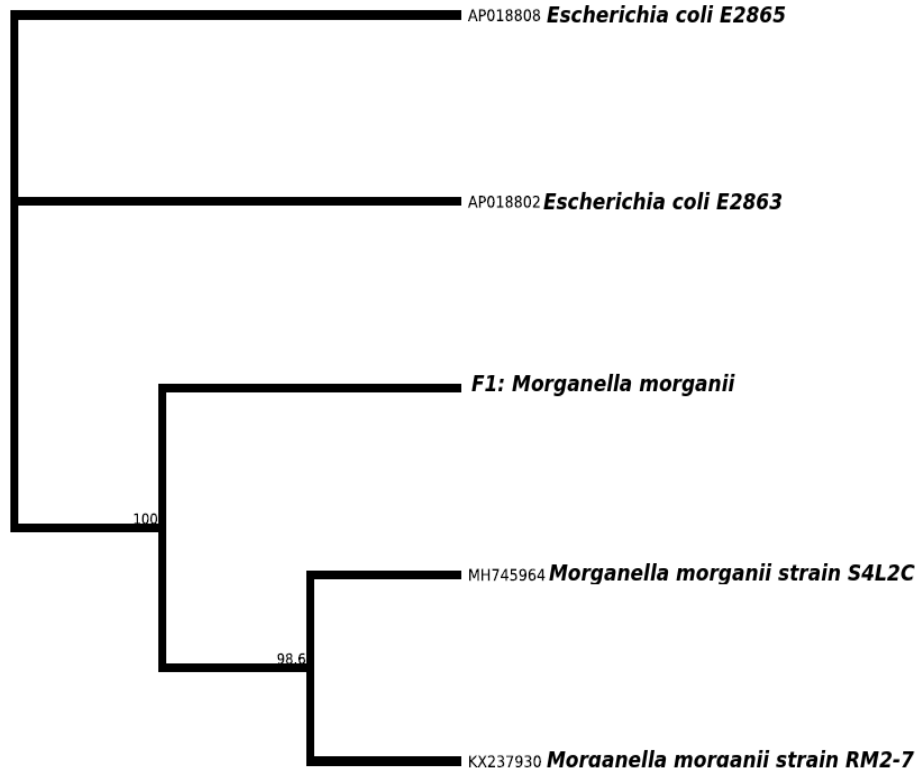


Fig. 1. Phylogenetic tree showing the evolutionary distance between the bacterial isolates

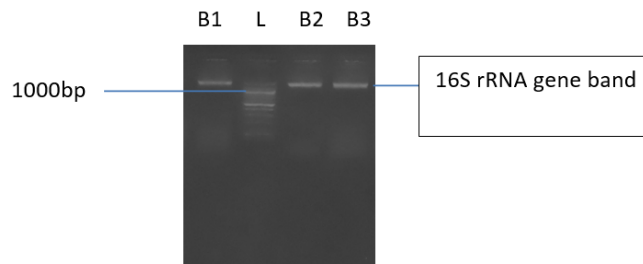


Fig. 2. Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates. Lanes B1, B2, B3 represent the 16SrRNA gene bands (1500bp), lane L represents the 100bp molecular ladder

Table 1. Chemical content determination of rice straw using isolated bacteria from termite gut

Anaerobic Digestion (AD) (Days)	Lignin %	Hemicelluloses %	Cellulose %
0	17.439	10.278	57.709
15	11.259	14.369	33.552
30	7.299	8.908	38.860

along the anaerobic digestion days. Zeng et al. [7] reported 29% of lignin removal from bamboo by *E. taxodii*2538 after 30 days of pretreatment. Chandra et al. 2007 reported about 37% of kraft

lignin degradation by bacteria species of *Novosphingobium* sp. B-7. 41.5% of kraft lignin was removed after 7 days of pretreatment by *Cupriavidus basilensis* B-8 [8] reported around

62% saccharification efficiency for agave atrovirens. Compared to the other lignolytic bacteria, *Morganella morganii* strain S4L2C (MH745964) isolated from termite guts has higher lignin-degrading capacity. Removal of lignin is a key factor for lignocellulose bioconversion process, since its existence hinders the availability of sugar monomers. The breaking of bonds that links polysaccharides to lignin leads to an easy way for microbial hydrolysis of the biotreated rice straw [7]. This, in turn, enhances the hydrolysis process. The contents of cellulose were increased by 36.12% after biotreatment. This change is due to structural disruption of rice straw caused by lignin depolymerization by *Morganella morganii* strain S4L2C (MH745964). In addition, 16.85% of hemicellulose was released after pretreatment. These bacteria reserve the cellulose and hemicellulose chains by selectively cutting lignin bonds. Compared to the content of lignin, cellulose and hemicellulose content of rice straw at charging a change in the major chemical composition of rice straw was observed after pretreatment. This change is mainly due to the solubilization of lignin by *Morganella morganii* strain S4L2C (MH745964). In this study, it was found that this bacterium has a potential lignin degrading ability to a greater extent and could play a major role in bioconversion of lignocellulose biomass to biogas.

#### 4. CONCLUSION

The study conducted on bio delignification and hydrolysis of rice straw showed that the significant capacity of bacteria isolated from wood feeding termite guts to remove rice straw lignin and increase hydrolysis efficiency. These showed the possibilities of biological conversion of lignocellulose bio-mass to its monomers by bacteria from termite guts. About 53.74% of rice straw lignin was removed after the 30<sup>th</sup> day of biotreatment. The cellulose and hemicellulose degrading abilities of the hydrolytic bacteria could have the advantage of increasing biogas yield. The separate delignification and hydrolysis were found to be a good combination to remove lignin

and to release the trapped cellulose and hemicellulose from rice straw.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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