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Molecular Identification of Electrogenic Bacteria Colonizing the Anode Electrode in Microbial Fuel Cell using Cow Urine as Inoculum

Aisha Salisu Buhari[1], Hindatu Yusuf[2]

[1]Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic Dutse. P. M. B 7040 - Nigeria
Email: *salisubuhariaisha[at]gmail.com*

[2]Department of Microbiology and Biotechnology, Faculty of Life Science, Federal University Dutse, 7156 Dutse Jigawa State, Nigeria
Email: *hindatyusuf2012[at]gmail.com*

Abstract: ***Electrogenic bacteria are group of microorganisms capable of transferring electrons extracellularly from their cell envelope to various electron acceptor, including electrodes. This study focused on the utilization of MFC to generate bioelectricity from wastewater using cow urine as inoculum and identify the bacteria colonizing the anode electrode. The samples were obtained from Dutse metropolis, Jigawa state, Nigeria. The voltage generated was measured using digital multimeter. The Bacterial biofilm formation on the anode was confirmed using scanning electron microscopy (SEM). The anodic bacteria were identified using molecular approach. The maximum voltage, power and current density obtained were 196 mV, 18.26 mW/m^2 and 97 mA/m^2 respectively. The SEM results showed that the bacteria were able to form biofilms attached to the electrode. The sequencing results showed the highest similarities to those of Bacillus paramycoides (98.69 %), Bacillus trophicus (99.87 %) and Priestia flexa (99.74 %). This result could contribute to improve understanding on the diversity and function of electrogenic bacteria for optimizing MFC performance and maximizing energy yield.***

Keywords: Electrogenic Bacteria, MFC, Electrode and Cow urine

1. Introduction

Current source of energy relied on fossils fuels used for transportation, power sector, waste management and in industries, which result in the emission of green house gases like carbon dioxide, thus contributing to global warming [1]. Alternative renewable energy has been developed in order to overcome the effect of this global worming resulting from the use of fossils fuels. Microbial fuel cell (MFC) have emerged as promising tool for sustainable waste treatment due to their ability to generate electricity through the metabolic activity of microorganisms [2]. MFC is a technology fed with microorganisms that mediate the conversion of chemical energy present in organic matter into electrical energy [3]. MFC is consist of two chambers separated by a proton exchange membrane (PEM), the exoelectrogenic bacteria oxidizes organic matter and produce electrons and protons. The electrons flows through the the external circuit whereas the PEM carries the protons to the cathode chamber. In the cathode chamber, protons and electrons reacted and combined with electrons acceptors such as oxygen and water is produced [2]. Electrogenic bacteria are group of microorganisms capable of transferring electrons extracellularly from their cell envelope to various electron acceptors, including minerals and electrodes, as well as to other bacteria. Microbes and electrodes interact electrochemically, which is the basis of the process [4]. This characteristic distinguishes them from other microorganisms that typically transfer electrons to internal electron acceptors, like oxygen or sulfate [5]. A prospective microbe needs to have adequate reducing power and a cellular mechanism that allows electrons to be transported from the live organism to the abiotic surface in order for this process to work [6]. MFC have an anodic surface where this electron transfer process can take place, which can be used to produce electrons. The specific electrogenic bacterial species present in cow urine utilizing wastewater from Dutse town have not been comprehensively identified and characterized. Understanding the diversity and function of these bacteria is crucial for optimizing MFC performance and maximizing energy yield. This research aims to bridge this knowledge gab by molecularly identifying the electrogenic bacteria isolated from MFC fed with wastewater from Dutse town, Nigeria.

2. Materials and Method

Sample Collection

Cow urine used as inoculum was collected from federal university dutse research and teaching farm, and it was prepared by transferring 2 ml in to 20 ml sterile nutrient broth and incubated at 37℃ for 24 h. This was used as biocatalyst during the MFC operation. While the wastewater used as substrate was collected using sterilized bottle from Gida - dubu area in Dutse, Jigawa state, Nigeria.

MFC Assembly and Operation

Two - chambered plastic MFC bottles with a capacity of 250 mL each and a working volume of 230 mL were used for the operation. The PEM (Nafion 117) was inserted between the chambers and it was fitted with a rubber gasket in order to prevent leaking. A 1.99 cm diameter plastic pipes was used to connect the chambers. Stainless steel mesh with a diameter of 4.4 cm and surface area of 38 cm^2 was used as the anode and cathode electrode. The two electrodes were connected by clamp with copper wire, and a resistor (1000 Ω) was connected across the circuit. The MFC reactor was sterilized prior to operation by rinsing it using 3% sodium hypochlorite solution and rinsed with distilled water, and then further

sterilized using 70% ethanol. The anode chamber was filled with 210 ml sterile substrate and 20 ml broth culture of cow urine as inoculum, and the cathode chamber was filled with 230 ml phosphate buffer and kept open to air to maintain an aerobic condition [7]. The MFC was operated via fed - batch mode spanning three cycle and readings were recorded at 24 h interval over 36 days. The control MFC was run by discarding the content of the MFC container after electricity generation, and sterilized as earlier stated, after which a fresh sterilized wastewater substrate was introduced without the cow urine. Voltage was measured at 12 h interval over a period of 15 days [8].

Measurement of Bioelectricity

The voltage was measured across a 1000 Ω external resistor every 24 h using digital multimeter (DT - 9205A). The voltage generated was employed to calculate the current (I) Eq.1, current density (CD) Eq.2 and power density (PD) Eq.3 using Ohm's law [5].

$$I = \frac{V}{R} \quad \text{Eq.1}$$

$$CD = \frac{I}{ASA} \quad \text{Eq.2}$$

$$PD = \frac{IV}{ASA} \quad \text{Eq.3}$$

Where; V = Voltage, R = Resistor and ASA is the anode surface area (cm^2)

Figure 1: Two chamber MFC utilized during the MFC operation

Scanning Electron Microscopy (SEM)

After the MFC operation, The electrode containing the microbial sample was removed from the MFC and rinsed with sterile distilled water to remove attached debris and 0.5cm^2 of the electrode was cut and immersed in 2.5% gluteraldehyde overnight to fixed the sample. Then the sample was dehydrated stepwise in a graded series of ethanol solution (30%, 50%, 70%, 85%, 95%) and then dried. Prior to viewing, the sample was coated with a layer of gold using sputtering [2].

Molecular Analysis

The surface of the anode electrode was scraped aseptically using sterile scalpel in 50 ml sterile distilled water and shaken to obtain biofilm suspension. The bacterial cells (at density of 1 x 10^9 cells/ml) were collected and centrifuged at 5009 xg for 5 min at 4 ℃. The Genomic DNA extraction was carried out using AccuPrep Genomic DNA Extraction Kit (K - 3032) from Bioneer according to the manufacturers instruction. The extracted DNA was amplified using universal forward primer–GGACTACAGGGTATCTAAT 16S (RIBOSE - 1), and reverse primer - AGAGTTTGATCCTGG 16S (RIBOSE - 2). PCR Amplification reaction was performed using PTC 100 thermal cycler. The PCR products were separated by electrophoresis using 1.5 % agarose gel for 35 min at 125 volt and then visualized the gel DNA bands using UV lightbox/ gel imaging system (Biorad). Amplified PCR products were sequenced at Inqaba Biotechnical Industries in South Africa. The sequencing results were submitted to BLASTn for sequence alignment and homology comparisons against the national center for biotechnology information (NCBI) GenBank database. The phylogenetic tree was constructed by MEGA11 based on the Maximum - likelihood method. The sequences obtained were deposited in the database [9].

3. Result and Discussion

Bioelectricity Generation

Figure 2 showed that, the MFC performance was recorded over the first 14 days for the first cycle with the highest voltage of 178 mV, second cycle was recorded over the next 10 days with maximum voltage of 196 mV and the third cycle was 183 mV with the addition of new substrate at the end of each cycle. The maximum power density achieved was 18.26 mW/m^2 following the achievement of the stable voltage while the maximum current density was 97.11 mA/m^2. This indicate that the voltage was mainly stable throughout the operation of MFC with gradual decrease toward the end of the operation. Generally, the electricity generated increased and reach its peak on the first few days after which it decreased gradually. This indicate that microorganism in the anode start to function effectively as soon as their nutrient source is replaced. This is in line with work of Jadhav *et al*., [10] using cow urine and sludge to generate electricity in MFC. The control MFC was also operated without the broth culture of cow urine, all operational parameters were the same with the experimental MFC, however, voltage reading was zero throughout the period of the experiment.

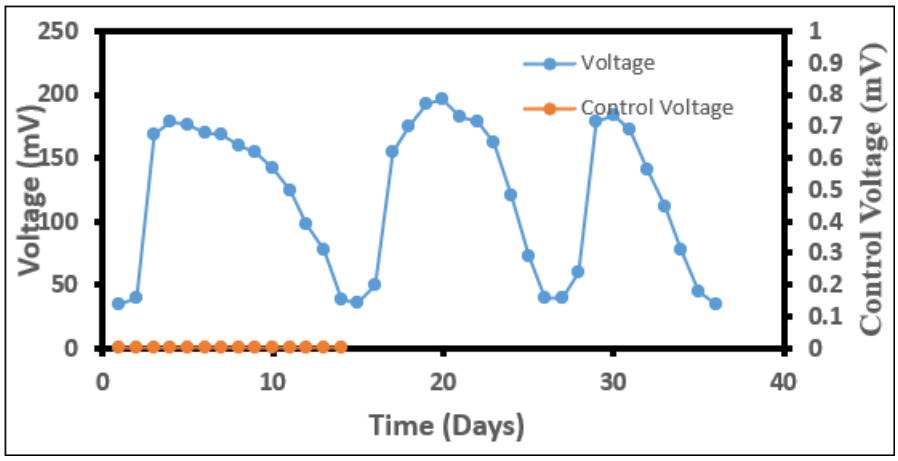

Figure 2: Voltage generated over 36 days of MFC operation using wastewater

Morphological Features of Biofilm Formation

Figure 3 shows the SEM morphological features of the biofilms attached to the electrode after electricity generation, recorded at a magnification of 8, 000x. The electrode's SEM image reveals a dense population of microorganisms on the stainless steel mesh electrode. The population is made up of long, short, and circular rod - shaped bacteria that have colonized the electrode's surface and are obviously involved in an electrochemical response. These results demonstrated that these strains are capable of producing electricity efficiently, which means that they can be effectively utilized to power the MFC.

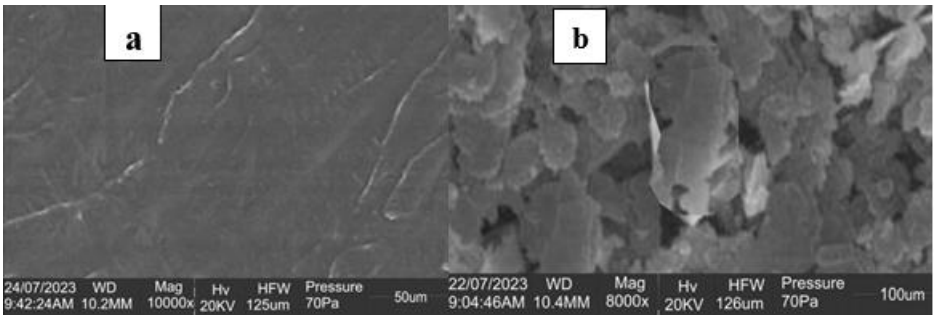

Figure 3: Morphological features of biofilms attached to the anode electrode (a) before MFC operation (b) after the MFC operation

Molecular Identification of the Bacterial Isolates

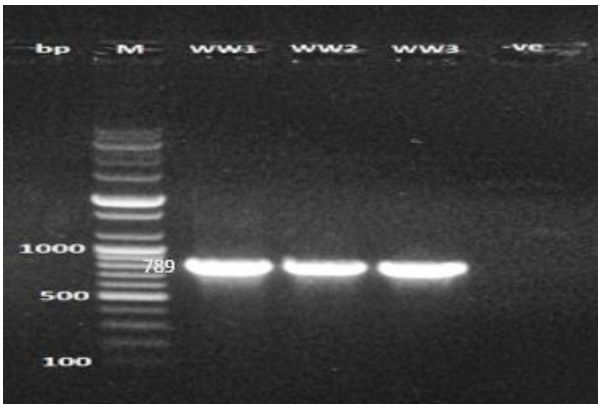

Plate 1: Gel Electrophoresis of Amplified PCR 16S rRNA Genes Bands of the Selected Isolates at 789 bp of the 100 bp plus DNA marker

Blast Analysis

Table 1 revealed that, the BLAST and phylogenetic tree analysis of the gene sequence from band WW1 (*Bacillus paramycoides* strain FUD - 006, accession number PQ269421) showed 98.69 % identity to *Bacillus paramycoides* strain MCCC 1A04098, accession number NR 157734 16S rRNA. Band WW2 (*Bacillus tropicus* strain FUD - 007, accession number PQ269422) showed 99.87 % sequence similarity to *Bacillus tropicus* strain MCCC 1A01406, accession number NR 157736 16S rRNA and Band WW3 (*Priestia flexa* strain FUD - 008, accession number PQ269424) showed 99.74 % sequence similarity to *Priestia flexa* strain IFO15715, accession number NR 024691 16S rRNA. *Bacillus paramycoides* and *Bacillus tropicus* are group of *Bacillus* spp in the family bacillaceae, biofilm formers and they were believed to be responsible for direct transfer of electron to the electrode in MFC [11]. These bacteria also have been reported to produce exopolysaccharide (EPS) in MFC which is an important step in biofilm matrix formation [12]. *Bacillus* spp isolated in this work were in line with the research of Onilude *et al.*, [13] using cow urine and wastewater, that identified *Bacillus* spp as electrogenic species. The *Bacillus* isolates proved to be promising isolates for application in bioelectricity generation using microbial electrochemical technologies. *Priestia flexa* is a bacterium recently discovered and is a novel bacteria to the field of MFC, the possible mechanisms that can make this organisms to be electrogenic bacteria is by direct attachment to the electrode due to the cell surface bound cytochromes, that help in bridging the distance between the cell wall and the electrode surface [14]. In general, the microbial diversity found in this study was higher than that found in other MFC study [15] which were dominated by *Thermincola* spp using marine sediment. This

distinction was probably attributable to the complex composition of the hydrolysate.

Table 1: Blast Analysis of the Selected Isolates

Band	Organisms	Searched Gene	Total Score	Query Cover	E value	Per Iden, (%)
WW1	*Bacillus paramycoides*	16S rRNA	1349	99%	0.0	98.69
WW2	*Bacillus tropicus*	16S rRNA	1393	97%	0.0	99.87
WW3	*Priestia flexa*	16S RNA	1395	98%	0.0	99.74

Keys: E Value = Expect value, Per Iden = percent Identity, ACC Len = Accesson length.

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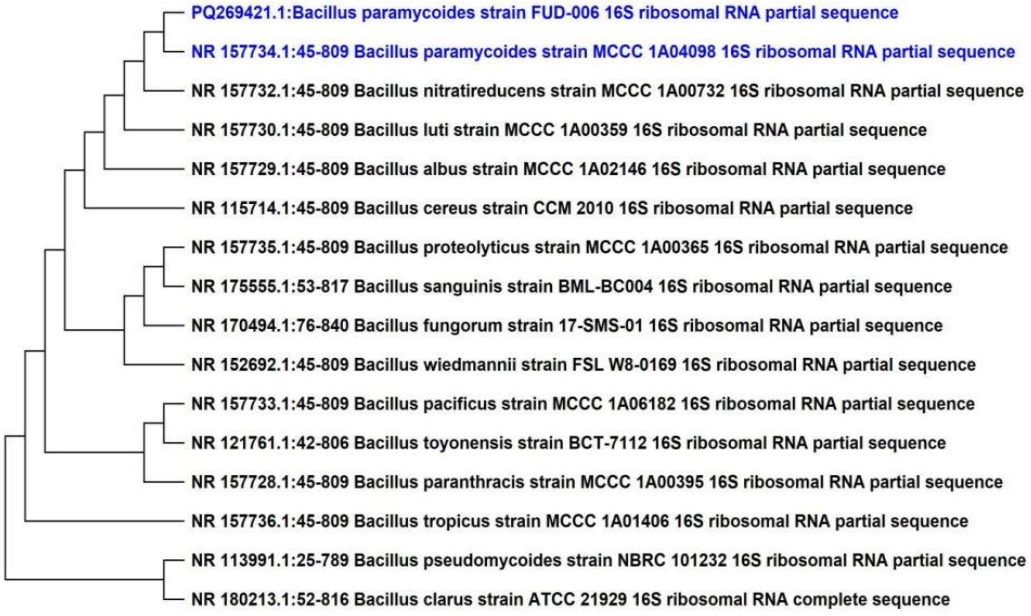

Figure 4: Phylogenetic Tree of W1 Isolate with Closely Related Strain Based on 16S rRNA Gene Analysis

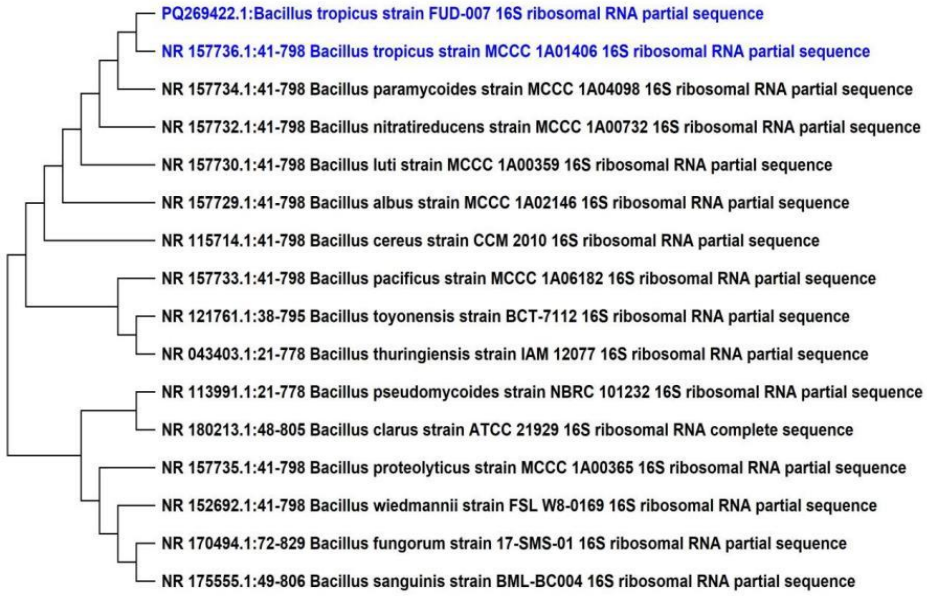

Figure 5: Phylogenetic Tree of W2 Isolate with Closely Related Strain based on 16S rRNA Gene Analysis

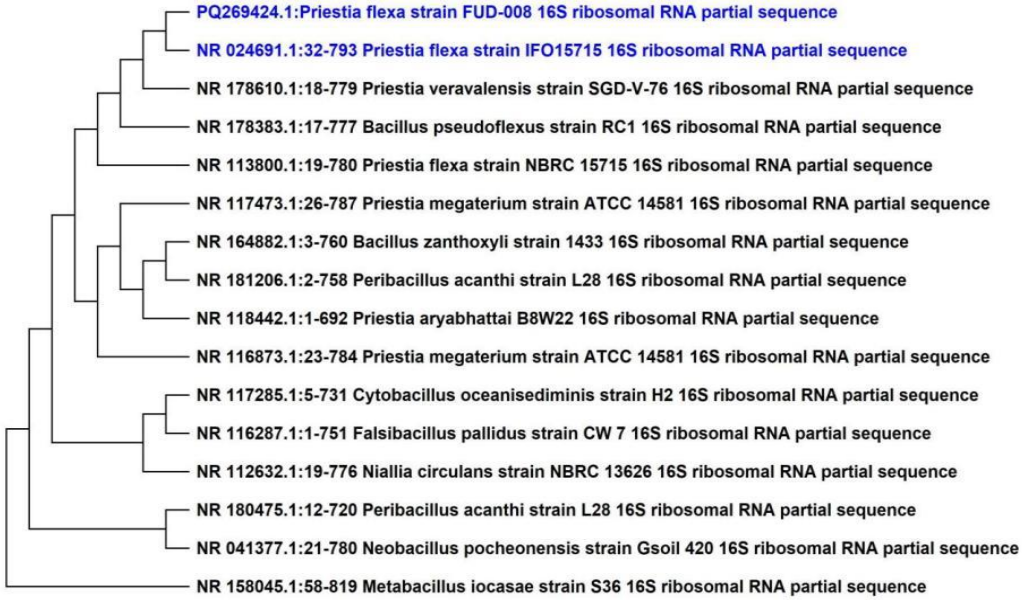

Figure 4.7: Phylogenetic Tree of W3 Isolate with Closely Related Strains based on 16S rRNA Gene Analysis

4. Conclusion

This study focused on using MFC to generate electricity from sewage wastewater using cow urine as inoculum. The maximum voltage of 196 mV, current density of 97.11 mA/m2, and power density of 18.26 mW/m2 were achieved. The molecular result showed the sequences similar to those of *Bacillus paramycoides (*98.69%), *Bacillus trophicus* (99.87%) and *Priestia flexa (*99.74%). This result could contribute to improve understanding on the diversity and function of electrogenic bacteria for optimizing MFC performance and maximizing energy yield.

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