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Bioelectricity generating potentials and molecular characterization of bacterial species from food processing wastewater using Microbial Fuel Cell Technology

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This study evaluates bioelectricity generating potentials of bacteria isolates from food processing wastewater. Fifteen bioelectricity-generating bacteria (BGB) were isolated from wastewater samples from five different food processing factories using ten-fold serial dilution and pour plate method. The isolates were identified based on Morphology and Biochemical characterization while the identities of the potent bioelectricity generating bacteria isolates were confirmed by 16SrRNA sequencing. The optimum growth temperature, incubation period and bioelectricity generation potential by the Bioelectricity generating bacteria were determined. Fifteen bacterial species were investigated for their potential as microbial catalysts based on their ability to metabolize locust beans wastewater as substrates. Inexpensive design of two chambered MFC incorporating operational parameters such as growth temperature, optical density, oxidizer, ion exchange membrane, anodic medium, electrode surface area and anodic volume cavity. All the bacteria screened for exoelectrogenic ability were able to produce voltage at minimal quantities. The statistical analysis revealed significant difference in voltage generation for period of fifteen days by some bacteria while voltage generation by others were not significantly different at $P < 0.05$. The eight bacteria with the highest voltage production: *Pseudomonas fluorescence*, *Pseudomonas taiwanensis*, *Pseudomonas putida*, *Myroides odoratimimus*, *Myroides gitamenses*, *Sphingobacterium mizutaii*, *Escherichia coli* and *Bacillus cereus* selected to investigate the effect of incubation temperature (20°C, 25°C, 30°C, 35°C, 40°C and 60°C) on voltage production had optimum growth of 0.53, 0.60, 0.45, 0.57, 0.31, 0.48, 0.52 and 0.56 at 30°C. The potential of each bacterium at their optimum temperature for electricity production (Voltage) showed that *Pseudomonas taiwanensis* had highest bioelectricity generation of 1.68V followed by *Pseudomonas fluorescence* (1.48V), *Sphingobacterium mizutaii* (1.43V), *Myroides gitamenses* (1.43V), *Pseudomonas putida* (1.42V) and the least voltage of 1.42 by *Escherichia coli*, *Bacillus cereus* and *Myroides odoratimimus*. The findings of this study revealed the abundance of bacteria equipped with essential ability to generate bioelectricity

Keywords: Bioelectricity, Voltage, Optical density, 16SrRNA sequencing

INTRODUCTION

Food industry processing wastewaters are abundant, rich in organic content which provides energy for bacterial cell to grow and have high biodegradability. It is therefore considered as an ideal substrate for electricity generation because Bacterial community depend on the composition, concentration and substrate types for higher output overall performance in energy production (Digman and Kim, 2008). A wide range of food industry waste waters have been reported for bioelectricity generation using microbial fuel cell including effluent from starch, whey, meat, vegetable, fish and waste streams from winery, brewery, dairy, canteen and other food processing industries (Velasquez-Orta et al. 2011).

Energy plays a crucial part in the day-to-day life starting from the origin of the universe. With the increasing energy demands, the effort has focused on developing low cost and efficient, energy harvesting technologies utilizing renewable energy sources. Energy structure of most countries, either developed or underdeveloped, depend on fossil fuels (Sugiawan and Managi, 2019). Their use has many problems in spite of their qualities, e.g., good operational control in thermal plants which have effect on the environments, scarcity, supply risk, and unsteadiness of prices and markets (Savvidis et al. 2019). The use of fossil fuels is responsible for environmental problems such as global warming and air pollution, which cause health problems and affect the quality of life of populations, also the scarcity increases the concerns about energy security due to their key role in today's energy production systems. However, non-renewability of fossil fuel raises the problem of their availability for this and future generations (Narula et al. 2017).

A fuel cell is a device that uses hydrogen as a fuel to produce electrons, protons, heat and water, the electrons can be harnessed to provide electricity in a consumable form through a simple circuit with a load (Chris et al. 2003). Microbial fuel cells are devices that employ microbial consortiums or single organism capable of generating electricity known as "electricigen" or "exoelectricigens" or "anode respiring bacteria" that have potential to metabolize organic compounds to carbon dioxide while directly transferring electrons to electrodes (Lovely, 2006). Different forms of bioenergy processes and technologies

are specifically linked with microbial communities (Wei et al. 2011 and Wang and Ren, 2013). Many studies have been found that MFCs contained diverse microbial communities in an MFC which includes *Shewanella putrefaciens* (Kim et al. 2002), *Rhodospirillum rubrum* (Chaudhuri and Lovley, 2003), *Geothrix fermentans* (Bond and Lovley, 2005) and *Escherichia coli*, *Proteus vulgaris* (Kim et al. 2007).

The study focuses on the isolation and molecular identification of bioelectricity generating bacteria from food processing wastewaters and evaluation of their potentials for bioelectricity production.

MATERIALS AND METHODS

Sample Collection

Five different sterile ten (10)-liter containers were used for the collection of all the substrate samples which are Brewery wastewater (BWW) obtained from International Breweries Plc Ilesa Osun State, Nigeria, Locust beans wastewater (LBWW) obtained from kolara area at idigba in Ogbomosho, Cassava wastewater (CWW) obtained from cassava processing unit of Arada market in Ogbomosho, Cow shed wastewater (CSWW) and sewage (S) was obtained from kara area Ogbomosho, Oyo State, Nigeria. The samples were collected into sterile kegs and transported to the laboratory for further analyses.

Isolation of electricity- generating Bacteria

Bacteria were isolated through plating from all the wastewaters. Waste water samples were serially diluted up to 10^{-10} . Dilutions of 10^{-3} , 10^{-6} , and 10^{-9} was used to inoculate nutrient agar plates using pour plate method and incubated at 37°C for 24 hours in order to isolate viable bacterial strains. The organisms were sub-culture subsequently until pure culture was obtained. Pure isolates were obtained using streaking method and stored in agar slants as stock cultures for further use and identification.

Microbial fuel cell (MFC) set-up and operation

Sixteen (16) two-chambered MFC were designed, eight (8) for waste waters both experimental set up and control in triplicate. A total volume of 1000 ml and working volume of 500 ml was filled with substrate and inoculum in the anode chamber while the cathode was filled

with oxidizer. Graphite rod of 15cm x 2cm dimension was used as electrode material. All the components of MFC were connected with proton exchange membrane internally and externally with wires from the electrode to the multimeter. Leakage test was carried out to check if the parts were properly fitted together during coupling and eventual loss of electrolytic fluids which can affect the successful operation. Therefore a quantity of clean water equivalent to the reactor capacity was then poured into the empty chambers and the system allowed standing for about one hour to ascertain the absence of leakage before the anolyte and catholyte were charged into the reactor. Also, the anode chamber environment was made completely anaerobic and cathode chamber was fully aerated. The MFC reactor set ups were surface sterilized prior to operation by 70% alcohol and dried UV light for 20 minutes (Kassongo and Togo, 2010) and kept at static conditions and voltage generation was recorded five (5) times daily for fifteen (15) days.

Screening of bioelectricity-generating Bacteria

Triplicates of dual chambered MFCs were set up alongside with control to study the bioelectricity production of each potential organism for fifteen (15) days. Locust beans wastewater was used as substrate and a loopful of different organism was inoculated into the different chamber. A total volume of 1litre and working volume of 500 ml of 0.1 M Potassium permanganate (KMnO_4), Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), phosphate buffer ($\text{K}_2\text{HPO}_4 + \text{KH}_2\text{HPO}_4$) and Potassium ferricyanide ($\text{K}_4\{\text{Fe}(\text{CN})_6\}3\text{H}_2\text{O}$) solutions were varied as catholyte in the cathodic chamber and same liquid volume for anolyte in the anodic chamber. Salt Agar Bridge (both KCl and NaCl) was used to connect anode and cathode chamber. Graphite electrode was inserted in both chamber and the voltage was measured five (5) times daily for fifteen (15) days using Multimeter. Potential organism with higher voltage were taken for further study.

Identification of the Potential Isolates

The identification was done on the basis of various staining procedures and biochemical tests was carried out at Kappa Biotechnology Laboratory (Research support, R&D and Analytical Services), Suites 19&20 Trans amusement Park, Old Airport, Bodija, Ibadan. Oyo State.

Effect of incubation temperature of some selected potential Bacteria on bioelectricity generation

The different values of bacteria) optical density (OD) were assessed at different growth temperature (20°C, 25°C, 30°C, 35°C, 40°C and 60°C) and used as inoculum at anodic chamber. A loopful of the organisms was inoculated into nutrient broth of 25 ml using slant bottles incubated for 24 hours. Optical densities of resulting suspension were measured at intervals by putting the samples in 0.5 cm cuvettes using UV spectrophotometer at 600 nm absorbance (OD_{600}). This was carried out at old biology laboratory of Ladoke Akintola University of Technology Ogbomosho. Oyo State, Nigeria.

Biofilm formation in bioelectricity production

Electrodes were carefully removed from the anodic chamber, heat fixed to allow the organisms to maintain its natural state and placed into a secured and well labelled chambers. It was further sent for SEM (scanning electron microscopy) and EDS/EDX (Energy Dispersed X-ray Spectroscopy) analysis were carried out before and after the experiment on the electrode to reveal the attachment and colonization of Bacteria cell because surface anode biofilm formation act as a biocatalyst in other to degrade the substrate and produce bioelectricity.

Molecular Characterization and phylogenetic tree of Selected Bacterial Isolates

The DNA of the isolates were extracted using the Bioscience Bacteria DNA Preparation Kit. Preceding to sequencing, the DNA was amplified using the polymerase chain reaction (PCR) technique in which universal primer (27F-AGAGTTTGATCCTGGCTCAG and 1492R-GGTTACCTTGTTACGACTT) were used. PCR products were purified and sequenced with a PCR purification kit. All PCR products were sent to Inquaba biotech (South Africa) for sequencing. Sequences were analyzed using BLAST from the National Center of Biotechnology Information (NCBI).

Statistical Analysis

Data obtained are by means of triplicate and analysis of the data were done by one way analysis of variance (ANOVA). Turkey's Multiple Comparison Test. Pearson correlation coefficient was determined and $p < 0.05$ were considered to be significant.



Figure 1: Microbial fuel cell set-up and operation

RESULTS AND DISCUSSION

Identification of potential bacteria isolates

The potential bacterial isolates are crucial in microbial fuel cell operation to know the activity of different organism (biocatalyst) present in wastewater. Fifteen Pure bacterial cultures were isolated from five different wastewaters (Table 1). Different species of bacteria which includes *Pseudomonas spp*, *Escherichia sp.*, *Proteus sp*, *Staphylococcus spp*, *Bacillus spp*, *Myroides sp*, and *Sphingobacterium sp*. isolated from these samples have also been reported to be dominant in food processing industries (Velasquez orta et al. 2011, Trond and Solveig, 2017).

Screening of bioelectricity-generating Bacteria

All the bacteria screened for exoelectrogenic ability were able to produce voltage at minimal quantities. The statistical analysis revealed significant difference in voltage generation for period of fifteen day by some bacteria while voltage generation by others were not significantly different at $P < 0.05$. The highest voltage yield as KCl oxidizer was used with phosphate buffer (PB), potassium dichromate (PD), potassium ferricyanide (PF) and potassium permanganate (PM) were 0.97V (*Bacillus cereus*), 0.71V (*Proteus vulgaris* and *Sphingobacterium mizutai*), 0.97V (*Bacillus polymyxa*) and 1.40V (*Bacillus licheniformis* and *Escherichia coli*) respectively

(Table 2). Also for NaCl as oxidizer, the highest voltage generated with PB, PD, PF and PM were 1.14V (*Pseudomonas taiwanensis*), 0.56V (*Bacillus Licheniformis*), 0.96V (*Pseudomonas fluorescence*) and 1.74V (*Pseudomonas putida*) respectively (Table 3). Potassium permanganate gave the highest performance with either KCl or NaCl as oxidizer. The operation carried out by Ghanapriya, 2012 depicted that an increase in electrical potential generation is associated with the oxidizer used (Park and Zeleus, 2003). He worked on 0.1M of three different oxidizer which are potassium permanganate solution, dichromate and potassium ferricyanide solution.

Effect of potential Bacteria at different incubation temperature on bioelectricity generation

The eight bacteria with the highest voltage production: *Pseudomonas fluorescence*, *Pseudomonas taiwanensis*, *Pseudomonas putida*, *Myroides odoratimimus*, *Myroides gitamenses*, *Sphingobacterium mizutaii*, *Escherichia coli* and *Bacillus cereus* were selected to investigate the effect of incubation temperature (20°C, 25°C, 30°C, 35°C, °C 40°C and 60°C) on voltage production. (Table 4). They had optimum growth of 0.53, 0.60, 0.45, 0.57, 0.31, 0.48, 0.52 and 0.56 respectively at 30°C.

Table 1: Bacteria isolates from wastewaters samples

wastewater	Isolates
Locust beans wastewater	<i>Bacillus sp.</i> and <i>Bacillus sp.</i>
Cassava wastewater	<i>Escherichia sp.</i> , <i>Proteus sp.</i> and <i>Pseudomonas sp.</i>
Brewery wastewater	<i>Bacillus sp.</i> , <i>Bacillus sp.</i> , <i>Bacillus sp.</i> , <i>Staphylococcus sp.</i> and <i>Bacillus sp.</i>
Cowshed wastewater	<i>Myroides sp.</i> , <i>Sphingobacterium sp.</i> and <i>Pseudomonas sp.</i>
Sewage wastewater	<i>Myroides sp.</i> and <i>Pseudomonas sp.</i>

Table 2: Screening for voltage generation of potential bacteria isolated when KCl based Agar Bridge was used with different oxidizer

Isolated Bacteria	KCl+ K ₂ HPO ₄ + KH ₂ HPO ₄ (PB)	KCl+ K ₂ Cr ₂ O ₇ (PD)	KCl + K ₄ [Fe(CN) ₆]3H ₂ O (PF)	KCl+KMNO ₄ (PP)
<i>Bacillus polymyxa</i>	0.67±0.00 ^e	0.49±0.00 ^e	0.97±0.0 ^d	0.96±0.00 ^e
<i>Bacillus megatarium</i>	0.83±0.00 ^{ab}	0.45±0.00 ^c	0.85±0.0 ^d	0.93±0.00 ^a
<i>Bacillus alvei</i>	0.77±0.01 ^a	0.51±0.00 ^b	0.49±0.20 ^a	0.91±0.42 ^d
<i>Bacillus subtilis</i>	0.62±0.00 ^a	0.57±0.00 ^f	0.75±0.00 ^f	1.00±0.00 ^a
<i>Bacillus licheniformis</i>	0.72±0.02 ^e	0.45±0.00 ^f	0.82±0.00 ^d	1.40±0.00 ^b
<i>Escherichia coli</i>	0.81±0.00 ^a	0.65±0.00 ^{cd}	0.64±0.00 ^b	1.40±0.00 ^g
<i>Bacillus cereus</i>	0.97±0.00 ^h	0.68±0.03 ^a	0.95±0.00 ^e	1.34±0.04 ^a
<i>Proteus vulgaris</i>	0.54±0.00 ^f	0.71±0.00 ^c	0.74±0.02 ^f	0.78±0.00 ^{hi}
<i>Pseudomonas Fluorescence</i>	0.80±0.00 ^b	0.50±0.00 ^{bed}	0.91±0.00 ^f	1.28±0.07 ^d
<i>Staphylococcus aureus</i>	0.68±0.04 ^c	0.60±0.01	0.62±0.02 ^c	0.83±0.00 ^{de}
<i>Pseudomonas taiwanensis</i>	0.78±0.00 ^g	0.48±0.00 ^e	0.70±0.20 ^a	0.72±0.07 ^f
<i>Pseudomonas putida</i>	0.56±0.00 ^a	0.42±0.00 ^b	0.42± 0.00 ^b	0.42±0.31 ^d
<i>Myroides odoratimimus</i>	0.90±0.01 ^h	0.48±0.00 ^c	0.66±0.00 ^h	0.81±0.00 ^{ghi}
<i>Myroides gitamenses</i>	0.55±0.00 ^g	0.56±0.00 ^d	0.51±0.00 ^c	0.92±0.00 ^c
<i>Sphingobacterium mizutai</i>	0.55±0.02 ^e	0.71±0.00 ^a	0.96±0.00 ⁱ	1.32±0.02 ^h
control	0.19±0.01 ^a	0.19±0.01 ^b	0.19±0.01 ^a	0.19±0.01 ^a

Values showed by different letters within and across the group showed that rate of bioelectricity generation are significantly different from each other statistically (P<0.05) WHILE those with the same letters are not significantly different (P<0.05) using Turkey comparison test. Values are given as mean values± standard deviation

Table 3: Screening for voltage generation screening of potential bacteria isolated when NaCl based Agar Bridge was used with different oxidizer

Isolated Bacteria	NaCl + K ₂ HPO ₄ +KH ₂ PO ₄ (PB)	NaCl+ K ₂ CrO ₂ (PD)	NaCl+ K ₄ [Fe(CN) ₆]3H ₂ (PF)	NaCl + KMNO ₄ (PP)
<i>Bacillus polymyxa</i>	0.71±0.00 ^a	0.51±0.00 ^{ef}	0.60±0.02 ^d	0.44±0.00 ^f
<i>Bacillus megatarium</i>	0.62±0.12 ^c	0.46±0.00 ^b	0.47±0.01 ^{def}	0.84±0.08 ^g
<i>Bacillus alvei</i>	0.78± 0.00 ^g	0.55±0.00 ^e	0.67±0.00 ^g	0.21±0.00 ^f
<i>Bacillus subtilis</i>	0.76±0.00 ^g	0.50±0.00 ^g	0.75±0.02 ^c	0.80±0.00 ^a
<i>Bacillus licheniformis</i>	0.55±.00 ^g	0.56±0.00 ^d	0.80±0.00 ^f	0.97±0.00 ^g
<i>Escherichia coli</i>	0.82±0.00 ⁱ	0.53±0.00 ^e	0.76±0.00 ^f	0.24 ±0.04 ^b
<i>Bacillus cereus</i>	0.84± 0.00 ^f	0.52±0.00 ^e	0.55±0.00 ^e	0.78 ±0.00 ^g
<i>Proteus vulgaris</i>	0.70 ±.00 ^f	0.53±0.00 ^g	0.78±0.02 ^f	0.88± 0.00 ^h
<i>Pseudomonas Fluorescence</i>	0.47±.00 ^h	0.54±0.00 ^e	0.96±0.00 ^b	1.08± 0.00 ^h
<i>Staphylococcus aureus</i>	0.75±.00 ^g	0.53±0.00 ^f	0.95±0.00 ^g	0.74±.00 ^c
<i>Pseudomonas taiwanensis</i>	1.14±0.00 ^f	0.51±0.00 ^c	0.66±0.00 ^{ef}	1.05±.00 ^k
<i>Pseudomonas putida</i>	0.55±.00 ^h	0.50±0.00 ^e	0.85±0.49 ^g	1.75 ±.00 ^g
<i>Myroides odoratimimus</i>	1.11±.00 ^g	0.53±0.00 ^c	0.86±0.00 ^d	1.05 ±.00 ^{gh}
<i>Myroides gitamenses</i>	0.53±.00 ^c	0.50±0.01 ^e	0.59±0.00 ^a	1.73±.00 ^{abcd}
<i>Sphingobacterium mizutai</i>	0.77±.00 ^h	0.49±0.00 ^d	0.88±0.00 ^c	1.34±.00 ^a
Control	0.19±0.01 ^e	0.19±0.01 ^a	0.19±0.0 ^a	0.19±0.00 ^a

Values showed by different letters within and across the group showed that rate of bioelectricity generation are significantly different from each other statistically (P<0.05) WHILE those with the same letters are not significantly different (P<0.05) using Turkey comparison test. Values are given as mean

values± standard deviation

Table 4: Growth Profile (OD_{600nm}) of Potential bioelectricity generating Bacteria at Different Temperature

(°C)	Temperature							
	MO	PT	EC	BC	PF	MG	SM	PP
20	0.28	0.22	0.26	0.26	0.22	0.32	0.28	0.35
25	0.34	0.31	0.36	0.31	0.50	0.26	0.42	0.33
30	0.57	0.6	0.52	0.56	0.53	0.31	0.48	0.45
35	0.55	0.54	0.27	0.49	0.50	0.29	0.37	0.42
40	0.34	0.26	0.28	0.29	0.50	0.31	0.31	0.24
60	0.35	0.25	0.31	0.17	0.33	0.28	0.14	0.15

Key: *Pseudomonas fluorescense*- PF

Pseudomonas taiwanensis- PT

Pseudomonas putida –PP

Myroides odoratimimus- MO

Myroides gitamenses-MG

Sphingobacterium mizutaii-SM

Escherichia coli- EC

Bacillus cereus- BC

Table 5: Voltage produced by each bacterium at their respective optimum optical density

Selected isolates	Cell volume(2ml) at OD ₍₆₀₀₎	Voltage (V)
<i>Escherichia Coli</i>	0.52	1.40
<i>Bacillus cereus</i>	0.52	1.40
<i>Myroides odoratimimus</i>	0.57	1.40
<i>Myroides gitamenses</i>	0.32	1.43
<i>Pseudomonas florescence</i>	0.53	1.48
<i>Pseudomonas taiwanensis</i>	0.60	1.68
<i>Sphingobacterium mizutaii</i>	0.48	1.43
<i>Pseudomonas putida</i>	0.45	1.42

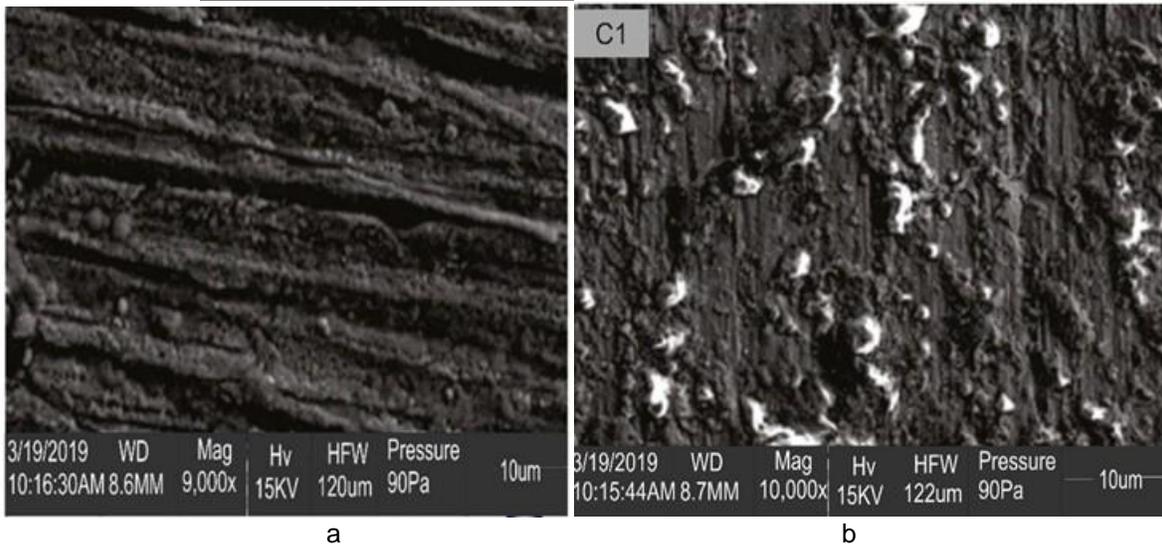
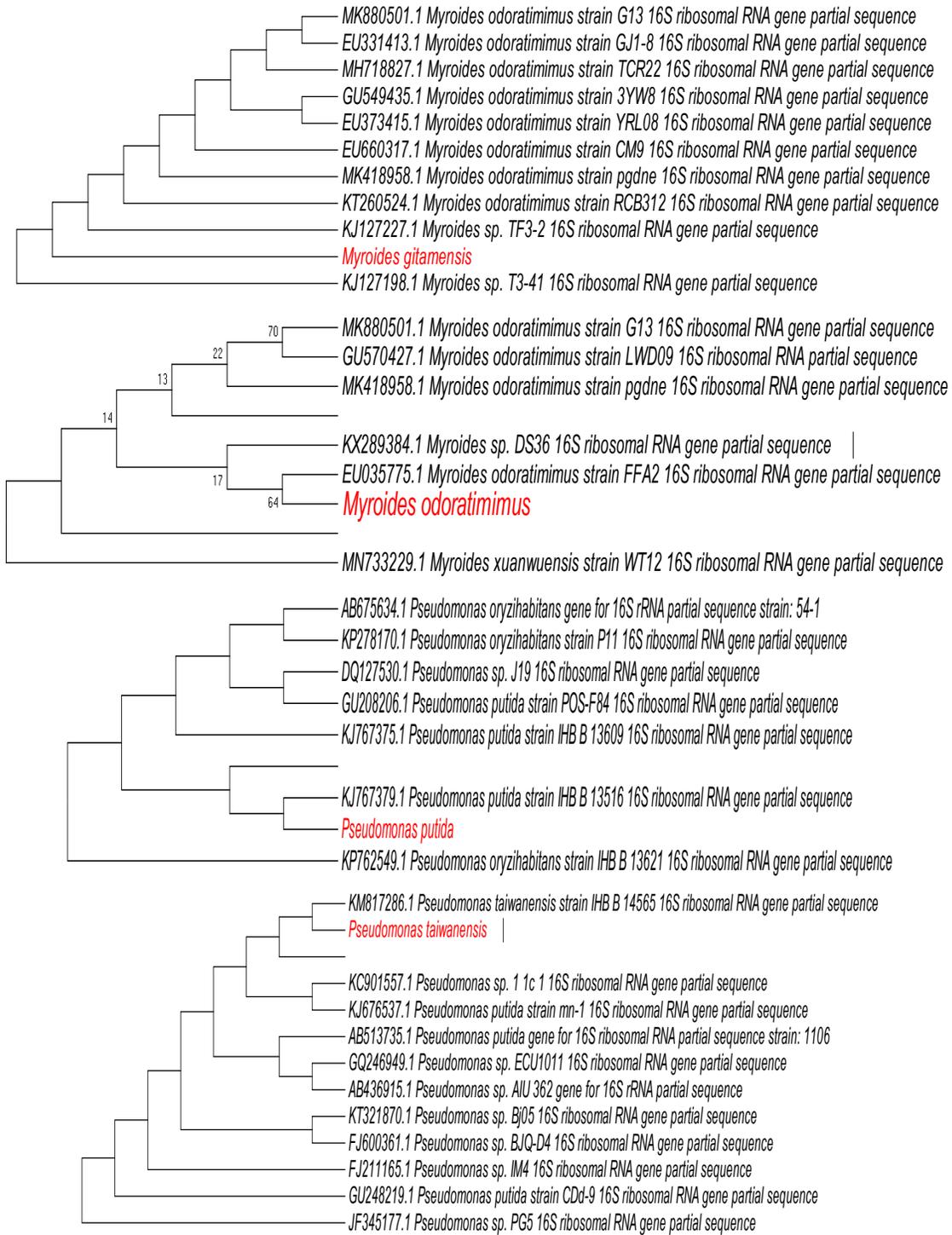


Figure 2a and b: Scanning Electron Microscope (SEM) before (a) and after (b) the experiment

The phylogenetic tree





The potential of each bacterium at their optimum temperature for electricity production (Voltage) showed that *Pseudomonas taiwanensis* had highest bioelectricity generation of 1.68V followed by *Pseudomonas fluorescence* (1.48V), *Spingobacterium mizutaii* (1.43V), *Myroides gitamenses* (1.43V), *Pseudomonas putida* (1.42V) and the least voltage of 1.42 by *Escherichia coli*, *Bacillus cereus* and *Myroides odoratimimus* (Table 5).

Jackie et al. 2011 reported that many organisms grow differently at different temperature. The growth rate and temperature are crucial in bacterial cultures. Bacteria can grow across a large spectrum of environmental conditions and basically three large groups which are Psychrophiles grow at 0-25°C at 25–40°C mesophiles and thermophiles at 40°C and above. This reports coincides with the findings of this work. *Pseudomonas taiwanensis* used in this study with 0.6 cell volume at OD₆₀₀ has the highest cell volume with the highest voltage generation which means the higher the value of OD the higher the voltage. Higher voltage exhibited by *Pseudomonas taiwanensis* compared to other isolates could be attributed to an increase in transfer of electron and proton to the electrode as a result of cell mass increase.

Scanning Electron Microscope (SEM)

The SEM of plain electrode showed no biofilm formation (figure 2a). Coverage of microbial attachment was abundant and evident on the electrode after the experiment when compared with the plain electrode surface (figure 2b). Microbial populations observed on the electrode plate under examination showed the presence of biofilm formation in this experiment which align with Rabaey and Rozendal, (2010) that power generation capacity is related to the biofilm formed on anodes as microbes adhere to their surface.

Molecular Characterization and phylogenetic analysis of the selected bioelectricity generating bacteria.

The five selected isolates DNA submitted to the NCBI database are *Myroides odoratimimus* strain AV1, *Pseudomonas taiwanensis* strain AV2, *Myroides gitamensis* strain AV3, *Pseudomonas putida* strain AV4, *Spingobacterium mizutaii* strain AV5, and were assigned accession numbers KX436990.1, KX436991.1, KX436992.1, KX436993.1 and KX436994.1 respectively.

CONCLUSION

The study established that bacteria indigenous to food processing industries are potential tools for bioelectricity generation. Five bacteria with remarkable generation activity were isolated from food processing industry. They were identified as based on 16S rDNA gene sequence analysis. Hence, these bacteria can be used in bioelectricity generation..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

OOO performed the experiments and wrote the manuscript. OJK designed the work. VOA and TOE carry out both Molecular Characterization and construct the phylogenetic tree, TFB and TOO helped with statistical analysis, arranged and reviewed the manuscript. All authors read and approved the final version.

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