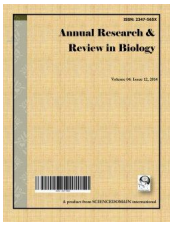

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Functional Screening of Electrogenicity and Bioelectricity Generation Potentials among Water-Borne Microbial Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OMA and AAO designed the study and wrote the protocol. Authors OMA, OAO and EOG managed the literature searches, conducted experimental analyses and laboratory procedures. Authors OMA and AAO performed the statistical analyses and wrote the manuscript first draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess natural electrogenicity and bioelectricity generation potentials of microbial isolates from water sites in Nigeria
Study Design: Sampling of various water sites and microbial isolation for subsequent electrogenic characterization of isolates and bioelectricity generation
Place and Duration of Study: Department of Microbiology, University of Ibadan, Ibadan, Oyo state, Nigeria between January 2014 to June 2015**.**
Methodology: Various water sites in Nigeria were assessed, and samples collected. Isolation and identification of bacteria and yeasts were carried out using standard techniques. Electrogenic screening of pure culture using Open Circuit Voltage (OCV) measurements in biolelectrochemical reactors and Cyclic Voltammetry were carried out. Bioelectricity generation measurements using multimeters and calculations of Voltage, Current, Power and Coulombic efficiency in Open and Closed circuit systems were calculated.

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Results: A total of 362 microorganisms (254 bacteria; 108 yeasts) were isolated and screened for electrogenicity. Samples from a River Benue site in Yola, Nigeria harboured the highest number of electrogenic isolates among all sites assessed. Sixty-five microorganisms elicited electrogenicity out of which 47 were bacteria and 18 were yeasts. Based on their electrogenic potentials, 7 of the most efficient isolates with electrogenic voltages >500 mV were further selected, and molecularly identified by 16S rRNA and ITS region gene analyses as *Pseudomonas aeruginosa* A4 (KX397030), *Pseudomonas aeruginosa* B3 (KX397029), *Enterobacter aerogenes* 102 (KX397032), *Pseudomonas* sp. B1 (KX397031), *Pseudomonas aeruginosa* 104, *Bacillus cereus* 101 (KX397028) and *Pichia kudriavzevii* 103 (KX397033). Cyclic voltammetry carried out on the isolates confirmed their electroactivity in comparison with a non-electrogenic *Escherichia coli* ATCC 25922 strain. Bioelectricity generation experiments showed that *P. aeruginosa* A4 was the most electrogenic strain, eliciting the highest current of 86.37 ± 14.52 mA/m^2. The least current was observed for the *Pichia kudriavzevii* 103 strain (19.22 ± 9.02 mA/m^2).
Conclusion: All isolates proved to be good electrogens and efficient candidates for optimising bioelectricity production.

Keywords: Open circuit voltage; wastewater; electroactivity; bioenergy.

1. INTRODUCTION

The ever increasing global energy demand and the increase in fossil fuel consumption have over the years brought up the twin issues of energy sustainability and environmental regression. These pertinent factors have driven the research for alternative and or complementary energy to fossil fuels [1,2]. The conventional drive for such energy sources has pushed forward the bioelectricity option [3].

Bioelectricity is a natural phenomenon that involves the generation, carriage or transfer of electronic charge from living system, a perfect example of which are microorganisms [4]. Electric charges within microorganisms are basically intracellular with electron flow just on the level of the internal part of cell membranes or mitochondria [5]. However, some unique microorganisms have evolved to release the electrons and subsequently electric charge extracellularly, and microorganisms possessing such ability to transfer electron outside their cells to an insoluble and sometime inert electron acceptor are referred to as electrogens [6]. The ability to convert these charges directly into electricity has elicited global research interests, and bioprospecting for efficient electrogenic strains are still underway. Diversity and environmental prevalence of these strains from various water sites as well as the biomechanisms of their occurrence within such sites are still being experimentally probed into [7].

A number of electrogenic species have earlier been identified. Park and Zeikus [8] as well as other researchers [7,9] explained that *Shewanella* and *Geobacter* spp have been the most predominantly researched upon. Given the versatile nature of the environment in which these organisms exist, it is hypothesized that there will be more unique strains available. This fact was corroborated by the work of Sacco et al. [10] who recently isolated a novel electrogenic bacterium *Dietzia* sp RNV-4 and determined its electrogenicity.

Though the full mechanisms of electrogenicity are still being investigated, it is hypothesised that determining the bioprospects of environmental sites for electrogenic strains can lead to a better understanding of the electromicrobiological diversity of those sites and improve our repository of electrogenic strains for further application in bioenergy [3]. Despite this view, not many research works have delved into the dynamics of specie-based electrogenicity yields with respect to different isolation sites. This work thus sought to isolate microorganisms from different water sites, and determine their electrogenicities in view of their bioelectricity generation potentials.

2. MATERIALS AND METHODS

2.1 Sample Collection, Isolation and Pure Culture Methods

Raw water and wastewater samples were collected from various environmental sites covering South West, South East and North East parts of Nigeria; industrial, agricultural and domestic wastewater effluents (Lagos 6^{o}36'6.617''N, 3^{o}21'5.35''E; Owerri 5^{o}29'20.612''N, 7^{o}1'3.316''E; Enugu

6°27'30.118''N, 7°32'46.998''E; Ibadan 7°22'39.128''N, 3°56'49.342''E and Yola 9°12'12.587''N, 12°29'43.403''E), River/stream /dam samples were River Benue in Yola 9°16'46.16''N, 12°27'29.48''E); Bodija stream 7°25'8.269''N, 3°54'6.1664''E and Awba dam in Ibadan 7°23'28.19''N, 3°54'59.99''E. Clean plastic containers were used for sample collection. Samples were then placed in ice packs and subsequently stored in the refrigerator at 4°C before microbial isolation. Isolation of bacteria and yeasts was carried out using nutrient agar and potato dextrose agar supplemented with 100 µg/mL ampicillin respectively.

2.2 Screening for Potent Electrogenic Isolates

Screening for electrogenic isolates was carried out [11] with modifications using a Microbial Electrochemical Reactor (MER) configuration. A standard dual-chambered MER was used in batch cultivation. The MER had 100 cm^3 anodic and cathodic chamber volume, and 42.53 cm^2 electrode (cathode/anode) surface area. Copper wire connections between the electrodes and a digital multimeter (model DM-87, HTC Instruments®) were used. Nafion® N-115 served as a proton exchange membrane, and acrylic glass (polymethyl metacrylate - PMMA) was used as a reactor chamber material. The PMMA reactor was sterilized [12] by treatment with 40% Hydrochloric acid, 75% ethanol and ultraviolet radiation (254 nm UV-C; dose – 5000 µW.s/cm^2) at an exposure distance of 10 cm for 30 minutes. A glucose minimal salt media [13] was prepared with the following composition: Glucose - 10g/L, $(NH_4)_2SO_4$ - 5 g/L, KH_2PO_4 – 1 g/L, K_2HPO_4 – 0.4 g/L, 0.01 M $MgSO_4$ and was sterilized at 121°C for 15 minutes, adjusted to pH 7.2. An inoculum preparation (10^2 cells) of each test isolate was prepared. 10 mL of the inoculum was introduced into 80 mL of earlier prepared sterile medium and dispensed aseptically into the anode chamber. Potassium ferricyanide solution (100 mM) was used as the catholyte. The open circuit voltage (OCV) readings were monitored using the digital multimeter at 12-hourly intervals for up to 120 hours. Electrogenic isolates were regarded as isolates that produced an OCV value of >100 mV after a 120hour run at room temperature (28±2°C) [14,15].

2.3 Identification of Microorganisms

Bacterial isolates were presumptively identified based on the different morphological and biochemical properties they exhibited including growth in different selective media. Identity was confirmed using molecular procedures in which bacterial genomic DNA were extracted and 16S rRNA gene analyses were carried out using 520F with sequence 5'-ATT GGG TGT AAA GCG -3' (forward primer) and 1061R with sequence 5' – CGG CAC GAG CTG ACG AC – 3' (reverse primer) [16].

Presumptive yeast identification was carried out using cultural and microscopic observation of morphological properties as determined by methods of Kurtzman et al. [17]. The PCR amplification of yeast DNA was carried out targeting the ITS regions of rDNA according to the method of White et al. [18].The gene was amplified using ITS1 and ITS4 standard primers (ITS1; TCC GTA GGT GAA CCT GCG G, and ITS4; TCC TCC GCT TAT TGA TAT GC).

Sequencing reactions (forward and reverse) were performed on the PCR amplicons using automated sequencer (Xcelris Biotech, India). Resultant sequences were further analysed using the Basic Local Alignment Search Tool (BLAST). Sequence similarities were determined (http://www.ncbi.nlm.nih.gov/blast/), and the organisms' molecular identities were known.

2.4 Electrochemical Analyses of Electrogens

For determination of cellular electrochemical signatures, Cyclic voltammetry (CV) was conducted on the microbial systems according to the procedure of Wang et al. [19]. Glassy carbon electrodes (3 mm thickness, 10cm length) with plastic insulator were kept overnight in 1M HCl, and subsequently immersed in sterile de-ionised water. Measurements of CV at 12 h were adopted for the determination of general electroactivity within the isolates. Electrochemical chambers (EC) of 500 mL working volume were used. Isolates were separately tested. Twenty milliliters of 8% inoculum of each isolate was and inoculated into 180 mL of sterile minimal salt media (to reach a 200 mL working volume). The glassy carbon electrode served as the working electrode (WE), while the reference electrode (RE) was Ag/AgCl connected with a 100 mM KCl salt bridge having a 3 mm glass membrane (Phadke Instruments, India). Platinum wire (0.1 mm) was used as the counter electrode (CE). The three electrodes were placed close to each other making sure they avoid contact. The WE

was attached with Platinum wire to a potentiostat (Digi Ivy Instruments). The RE and CE were directly connected to the potentiostat, and the system was maintained at room temperature. Non-electrogenic *Escherichia coli* ATCC 25922 served as control in CV experiments. CV analyses were run at 10 mV/s with the WE poised at 400mV, and a potentiostat range of -1V to 1V. With the incubation of test isolates within the ECs for 12 hours, the CV of samples were performed for confirmation of electrogenicity of the isolates. Values of current (µA) and Potential Vs Ag/AgCl (Volts) were plotted on the Y and X axis respectively showing electrochemical activity and represented as cyclic graphs with oxidation and reduction curves.

2.5 Bioelectricity Measurements

Bioelectricity measurements of MER systems of each isolate were made by taking open and closed circuit voltage readings using a digital multimeter, and a 100Ω resistor. To determine the current, Ohm's law (Voltage = Current x Resistance) was used. Voltage plots against reaction time (hours) were measured and used as the volume of bioelectricity generated [20]. Power was determined by multiplying the value of the Voltage (Closed Circuit Voltage, CCV) and current measured [3].

Current density (mA/m^2) and Power density (mW/m^2) values were then deduced [3] as stated in the formulae below of which the maximum values (Current density $_{max}$ and Power density $_{max}$) was recorded for each isolate.

Current density (mA/m^2)

$$= \frac{\text{Current generated (mA)}}{\text{Anode surface area } (m^2)}$$

Power density (mW/m^2)

$$= \frac{\text{Power generated (mW)}}{\text{Anode surface area } (m^2)}$$

Coulombic efficiency (C.E in %) of each MER system was deduced by dividing the recovered Coulomb (C) by total Coulomb and multiplying by 100 [21]. One ampere of current was equivalent to 1C generated per second. Total Coulomb was calculated from the carbon source used in the media by multiplying the moles of electron per mole of substrate (carbon source) by Faradays constant (96,485 C/mol* electron) and substrate concentration divided by the molecular weight of the substrate. The C.E implied the efficiency of energy harvesting from the substrate. Energy produced was an equivalent of the product of maximum power in Watts generated and time taken in seconds [22].

3. RESULTS AND DISCUSSION

3.1 Isolation, Electrogenic Screening and Identification of Microorganisms

A total of 362 microorganisms consisting of 254 bacteria and 108 yeasts were isolated during the course of this research work covering various geographical locale in Nigeria. All sources harboured microorganisms in varying numbers. After screening, Sixty five (65) isolates (bacteria and yeasts) representing about 18% of the total number of microorganisms obtained from the various sites was determined as electrogenic while the rest (82%) were non-electrogenic (Tables 1 and 2).

Based on the screening and selection process adopted, the most electrogenic bacterial isolates (giving an electrogenic voltage >500mV) were *Pseudomonas* sp SBS4-A4, *Pseudomonas* sp SDW4-B3, *Pseudomonas* sp NRB20-B1, *Enterobacter* sp SDW16-102, *Pseudomonas* sp SUW7-104, and *Bacillus* sp. NMW13-101. Sequence analyses of their 16S rRNA genes, sequence comparisons on the NCBI website and, subsequent phylogenetic relationships (Fig. 1) confirmed the isolates' identity. Isolate SBS4-A4 was identified as a *Pseudomonas aeruginosa* A4 and after sequence upload, it was allotted an accession number of KX397030. Similar procedure was also followed for the other bacterial isolates. Isolate SDW4-B3 as *P. aeruginosa* B3 (KX397029), isolate SDW16-102 identified as *Enterobacter aerogenes* 102 (KX397032), isolate NMW13-101 was identified as *Bacillus cereus* 101 (KX397028), isolate NRB20-B1 identified as *Pseudomonas* sp B1 (KX397031), and Isolate SUW7-104 was also fully identified as *P. aeruginosa* 104 (Accession number yet to be obtained). The best yeast isolate BWE9-103 (producing >500mV at screening) had a morphology similar to *Candida* species. This strain with further molecular analysis was confirmed to be *C. krusei* which had undergone reclassification as an *Issatchenkia orientalis* and subsequently *Pichia kudriavzeii* – from phylogenetic comparison as shown in Fig. 2. The isolate was named *P. kudriavzevii* 103 (KX397033)

Table 1. Overview of number of electrogenic microorganisms isolated and isolates' sources within Nigeria

S/N	Isolates source	Isolate code	Total number of isolates from source	Number of electrogenic isolates	Number of electrogenic Yeasts isolated	Number of electrogenic Bacteria isolated
1.	Brewery Waste water (Enugu, South East)	BWE	19	4	3	1
2.	Domestic waste water (Lagos, South West)	DWL	12	2	1	1
3.	Domestic waste water (Owerri, South East - A)	DWO	18	3	1	2
4.	Animal farm house effluent (Owerri, South East)	FHO	13	1	-	1
5.	Animal farm house effluent (Lagos, South East)	FHL	10	1	1	-
6.	Industrial waste effluent A (Lagos, South West)	ILA	15	1	1	-
7.	Industrial waste effluent B (Lagos, South West)	ILB	9	-	-	-
8.	Industrial waste effluent C (Lagos, South West)	ILC	13	-	-	-
9.	Domestic waste water (Owerri, South East – B)	DWO2	10	-	-	-
10.	Domestic waste water (Lagos, South west –B)	DWL2	13	-	-	-
11.	Municipal waste water (Owerri, South East)	OMW	15	-	-	-
12.	Municipal waste water (Lagos, South East)	LMW	14	1	-	1
13.	Bodija stream (Ibadan, South West)	SBS	22	7	1	6
14.	River Benue (Yola, North East)	NRB	24	13	5	8
15.	Domestic waste water (Yola, North east)	NDW	26	3	1	2
16.	Domestic waste water (UI	SUW	19	7	1	6

S/N	Isolates source	Isolate code	Total number of isolates from source	Number of electrogenic isolates	Number of electrogenic Yeasts isolated	Number of electrogenic Bacteria isolated
	Ibadan, South west)					
17.	Municipal waste water (Yola, North East)	NMW	38	6	2	4
18.	Oba Dam water (UI Ibadan, South west)	SMW	33	3	-	3
19.	Domestic waste water (Ibadan, South west)	SDW	22	8	1	7
20.	Domestic Waste water (MAUTECH Yola, North east)	NUW	17	5	-	5
			362	**65**	**18**	**47**

Table 2. Data on screened electrogenic isolates based on amount of electrogenic potentials in descending order

Isolate's code	Source	*Open circuit voltage (mV)	Status remark
SBS4-A4	Bodija stream, Ibadan, Nigeria	556.03 ± 1.21	Electrogenic bacteria
SDW4-B3	Domestic waste water, Lagos, Nigeria	554.11 ± 0.35	Electrogenic bacteria
SDW16-102	Domestic waste water, Ibadan, Nigeria	550.24 ± 0.23	Electrogenic bacteria
NRB20-B1	River Benue, Yola, Nigeria	548.91 ± 1.30	Electrogenic bacteria
SUW7-104	Domestic waste water, University of Ibadan Campus, Nigeria	545.23 ± 1.75	Electrogenic bacteria
NMW13-101	Municipal waste water, Yola, Nigeria	540.95 ± 0.33	Electrogenic bacteria
BWE9-103	Brewery waste water, Enugu, Nigeria	528.62 ± 0.56	Electrogenic yeast
SMW20	Oba Dam water, University of Ibadan	496.12± 0.32	Electrogenic bacteria
NRB9	River Benue, Yola, Nigeria	495.14± 0.71	Electrogenic bacteria
SBS11	Bodija stream, Ibadan, Nigeria	493.54± 1.11	Electrogenic bacteria
SDW13	Domestic waste water, Ibadan, Nigeria	490.32± 1.21	Electrogenic bacteria
NMW1	Municipal waste water, Yola, Nigeria	486.65± 0.68	Electrogenic bacteria
NRB16	River Benue, Yola, Nigeria	485.38± 0.93	Electrogenic bacteria
NRB5	River Benue, Yola, Nigeria	483.75± 0.54	Electrogenic yeast
NUW5	Domestic waste water, MAUTECH campus, Yola Nigeria	478.34± 0.63	Electrogenic bacteria
NRB14	River Benue, Yola, Nigeria	474.44± 0.38	Electrogenic bacteria
SUW11	Domestic waste water, University of Ibadan Campus, Nigeria	470.31± 0.87	Electrogenic bacteria
NDW11	Domestic waste water, Yola, Nigeria	470.76± 1.12	Electrogenic yeast

Isolate's code	Source	*Open circuit voltage (mV)	Status remark
NDW12	Domestic waste water, Yola, Nigeria	470.05± 1.71	Electrogenic bacteria
NUW7	Domestic waste water, MAUTECH campus, Yola Nigeria	466.06± 1.04	Electrogenic bacteria
NRB3	River Benue, Yola, Nigeria	451.84± 0.36	Electrogenic bacteria
SMW16	Oba Dam water, University of Ibadan	444.54± 0.39	Electrogenic bacteria
SBS8	Bodija stream, Ibadan, Nigeria	439.54± 0.45	Electrogenic bacteria
SDW1	Domestic waste water, Ibadan, Nigeria	438.51± 0.72	Electrogenic bacteria
NRB18	River Benue, Yola, Nigeria	438.19± 0.91	Electrogenic yeast
NRB1	River Benue, Yola, Nigeria	434.43± 0.66	Electrogenic bacteria
SBS3	Bodija stream, Ibadan, Nigeria	430.26± 0.72	Electrogenic yeast
NRB6	River Benue, Yola, Nigeria	426.31± 0.43	Electrogenic bacteria
NMW8	Municipal waste water, Yola, Nigeria	423.37± 1.76	Electrogenic yeast
SBS18	Bodija stream, Ibadan, Nigeria	420.44± 1.65	Electrogenic bacteria
SDW3	Domestic waste water, Ibadan, Nigeria	411.12± 2.11	Electrogenic bacteria
SDW12	Domestic waste water, Ibadan, Nigeria	410.68± 0.67	Electrogenic bacteria
SUW13	Domestic waste water, University of Ibadan Campus, Nigeria	410.43± 0.84	Electrogenic bacteria
SUW6	Domestic waste water, University of Ibadan Campus, Nigeria	409.32± 0.52	Electrogenic bacteria
SMW5	Oba Dam water, University of Ibadan, Ibadan, Nigeria	403.45± 0.49	Electrogenic bacteria
SUW9	Domestic waste water, University of Ibadan Campus, Nigeria	399.41± 0.53	Electrogenic yeast
NMW12	Municipal waste water, Yola, Nigeria	377.22± 0.41	Electrogenic yeast
SBS16	Bodija stream, Ibadan, Nigeria	376.24± 0.78	Electrogenic bacteria
SBS13	Bodija stream, Ibadan, Nigeria	368.23± 0.83	Electrogenic bacteria
NMW13	Municipal waste water, Yola, Nigeria	363.34± 0.99	Electrogenic bacteria
NMW24	Municipal waste water, Yola, Nigeria	357.35± 0.82	Electrogenic bacteria
NRB12	River Benue, Yola, Nigeria	349.26± 0.77	Electrogenic yeast
SUW1	Municipal waste water, Ibadan, Nigeria	344.44± 2.11	Electrogenic bacteria
NUW14	Domestic waste water, MAUTECH campus, Yola Nigeria	336.36± 0.73	Electrogenic bacteria
SDW14	Domestic waste water, Ibadan, Nigeria	333.54± 0.86	Electrogenic yeast
NUW15	Domestic waste water, MAUTECH campus, Yola Nigeria	331.46± 0.48	Electrogenic bacteria
SUW6	Domestic waste water, University of Ibadan Campus, Nigeria	280.43± 0.52	Electrogenic bacteria
NRB21	River Benue, Yola, Nigeria	280.18± 0.67	Electrogenic yeast
NDW23	Domestic waste water, Yola, Nigeria	276.07± 0.62	Electrogenic bacteria
NUW15	Domestic waste water, MAUTECH campus, Yola Nigeria	275.00± 0.77	Electrogenic bacteria
SDW7	Domestic waste water, Ibadan, Nigeria	269.41± 0.69	Electrogenic bacteria
LMW25	Municipal waste water, Lagos, Nigeria	262.32± 0.81	Electrogenic bacteria

Isolate's code	Source	*Open circuit voltage (mV)	Status remark
BWE11	Brewery waste water, Enugu, Nigeria	230.90± 0.54	Electrogenic bacteria
DWO23	Domestic waste water, Owerri, Nigeria	217.21± 1.29	Electrogenic bacteria
DWO20	Domestic waste water, Owerri, Nigeria	204.32± 2.11	Electrogenic yeast
FHL11	Animal farm house effluent, Lagos, Nigeria	198.45± 0.65	Electrogenic yeast
BWE6	Brewery waste water, Enugu, Nigeria	190.45± 0.07	Electrogenic yeast
NRB27	River Benue, Yola, Nigeria	181.83±0.82	Electrogenic yeast
DWO3	Domestic waste water, Owerri, Nigeria	173.54± 0.66	Electrogenic bacteria
FHO5	Animal farm house effluent, Owerri, Nigeria	157.63± 0.62	Electrogenic bacteria
ILA8	Industrial waste water effluent, Lagos, Nigeria	133.49± 0.18	Electrogenic yeast
DWL1	Domestic waste water , Lagos, Nigeria	123.41± 0.87	Electrogenic bacteria
NRB29	River Benue, Yola, Nigeria	118.42± 0.65	Electrogenic bacteria
BWE7	Brewery waste water, Enugu, Nigeria	108.80± 0.77	Electrogenic bacteria
DWL4	Domestic waste water , Lagos, Nigeria	103.00±0.61	Electrogenic yeast

**values of voltage (mV) are represented as Mean±SD (standard deviation)*
*Isolate **codes in bold** indicate the isolates selected for further analyses*

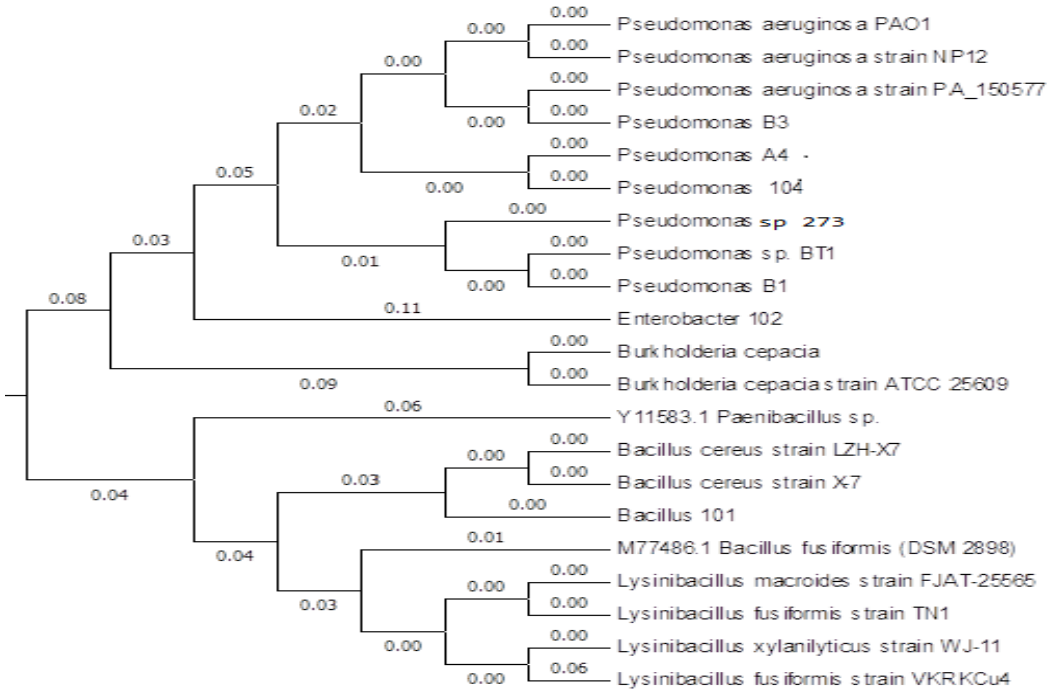

Fig. 1. Phylogenetic tree of electrogenic bacterial isolates with closely related strains based on 16s rRNA gene analyses

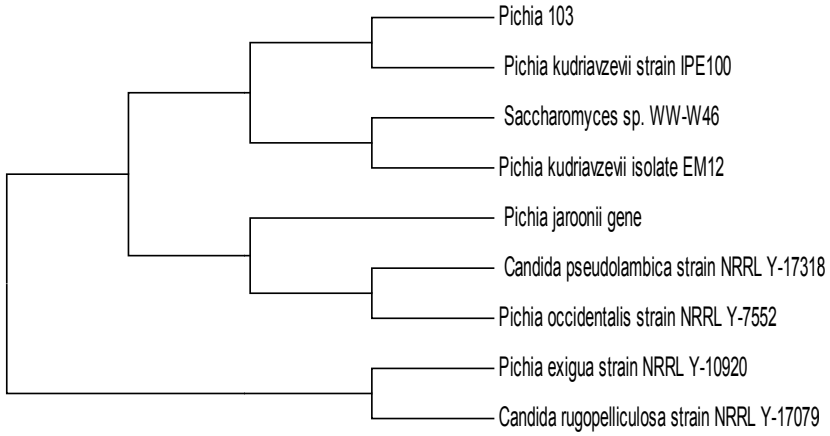

Fig. 2. Phylogenetic comparison of sequence of electrogenic *Pichia* sp. 103 with closely related yeast strains based on the amplification of the ITS regions

The ecological/ environmental presence and diversity of electrogenic microorganisms are still being studied the world over [23]. Results of screening of the microorganisms isolated in this work for electrogenicity showed that 18% out of the total number isolated proved to be electrogenic. Similar range of prevalence has been corroborated by previous research [3]. The screening procedure for electrogenic qualification of microorganisms isolated was based on their abilities to elicit electrogenic voltages which were quantified as Open Circuit Voltages (OCV) [11,24]. The most electrogenic ones which yielded electrogenicity of >500mV were selected for further experimentation.

Electrogenicity in microorganisms is not a peculiarity of any known genera of microorganisms alone, rather it is hypothesized to be spread among genera found in a variety of prevalent environmental species [25]. Phung et al. [25] also stated that wastewater bodies and other natural/artificial percolation of environmental water have been important repositories for electrogenic microbial species, after isolating electrogenic proteobacteria and other classes from waste water samples. Lee et al. [26], Kim et al. [27] and Kumar et al. [28] all corroborated this concept as they worked on the functional presence of electrogenic microorganisms within water as an isolation source. All isolates in this work were from water bodies and water-laden industrial/domestic waste effluents. There was conformity in percentage of organisms isolated in this work in comparison with earlier stated researches. Electrogenic bacteria isolated from this work were of the genera *Bacillus, Pseudomonas, Enterobacter, Aeromonas, Klebsiella,* and *Enterococcus*. However, the most electrogenic strains which also had their identities molecularly confirmed were from the genera *Pseudomonas*, *Bacillus* and *Enterobacter*. Electrogenic species among the yeasts isolated include the genera *Dabaromyces*, *Pichia*, *Candida*, *Rhodotorulla,* and *Saccharomyces.* However*, Pichia* sp. with electrogenicity >500mV, was selected for further research.

Pseudomonas species were the most electrogenic species as 22 of the 65 electrogenic microorganisms screened were identified as *Pseudomonas*. Previous researches have also classed *Pseudomonas* species as viable electrogens [19,29,30]. The unique metabolism of *Pseudomonas* and its electrogenic dexterity could be resultant from a number of reasons, ranging from high biodegradative and fermentative potentials to extensive environmental survival abilities, as well as high genetic capabilities [31]. *P. aeruginosa* strain A4 was identified as the most efficient electrogenic strain among all electrogenic *Pseudomonas* as well as among all electrogenic isolates identified. Investigations of some other *P. aeruginosa* strains along with strain A4 showed that there was variability in yield of electrogenicity across even strains of the same species. This could be attributed to physiological and genetic variances occurring within strains [32]. According to earlier research [33], the mechanisms of electrogenicity elicited by *Pseudomonas*, with particular reference to *P. aeruginosa* showed that electrogenicity was mediated by extracellular electroactive metabolites which possessed specific electrochemical signatures which could also vary based on highly genetic factors.

An *Enterobacter* sp was investigated in this work, and was further speciated as an *E. aerogenes.* The strain which was an efficient bioelectricity producer and the isolation of electrogenic *Enterobacter* is in concurrence with earlier reports by Feng et al. [34] who attempted characterising the electrochemistry of *Enterobacter* electrogenic system under Cospper shock load. Onilude et al. [30] had also assessed the electrogenic potentials of *Enterobacter* species using a bioelectricity generation system. A *Bacillus* sp identified as a *B. cereus* strain with a good electrogenic yield was also isolated. The determination of *Bacillus* species as electrogenic was also in agreement with reports by Shankar et al. [35] who investigated the electrogenic potentials of *Bacillus* species fed with cellulose for bioelectricity generation as well as simultaneous cellulose hydrolysis. Another research by Deepika et al. [36] had also identified a *Bacillus tequilensis* strain as an effective electrogen. The strain was also applied with a *Pseudomonas aeruginosa* in co-culture for bioelectricity generation.

The electrogenic yeast *Pichia* species isolated in this work is in agreement with reports by Wu et al. [37]. The *Pichia* sp was taxonomically classified as *Pichia kudravzevii* (also known as *Issatchenkia orientalis),* and identified as an anamorph of *Candida krusei*. This is the first report specifically implicating *P. kudriavzevii* as an electrogen, however, the occurrence of this strain further buttresses the point that there is still room for exploitation of natural electrogenic strains that have not been fully identified and characterised. With respect to yeasts, Arbianti et al. [38] had also correlated activity of yeasts as electrogenic microorganisms in bioelectricity systems.

3.2 Electrochemical Analyses of Electrogenic Isolates

Redox-positive activities as depicted by their cyclic voltammograms at the scan between -1V and 1V poised voltage potentials was exhibited by all the electrogenic isolates. There were evident oxidation and reduction cycles which implied the presence of reduced and oxidized chemical species within the electrogenic systems of each test isolate. As shown in figure 3 all the

strains were electroactive within their test systems thus indicating the effects of possible redox mechanisms elicited by the electrogenic strains. There was also a clear distinction in the cyclic voltammogram of a non-electrogenic *E. coli* strain (ATCC 25922) (negative control strain) in comparison with test electrogens. There was a very negligible gap between the reduction and oxidation curves for the cycle of the negative control strain. This thus confirmed its non-electrogenic/non-electroactive status.

Similarities in the cyclic voltammogram curve patterns between the cyclic plots for *B. cereus* 101 and *P. kudriavzevii* 103 electrogenic isolates suggested a similarity in electroactive mechanisms between the two organisms. A trend was also observed in the pattern similarity between the cyclic voltammogram plots of all the *Pseudomonas* species tested. This also pointed to a similarity in electroactive mechanisms adopted by the *Pseudomonas* species.

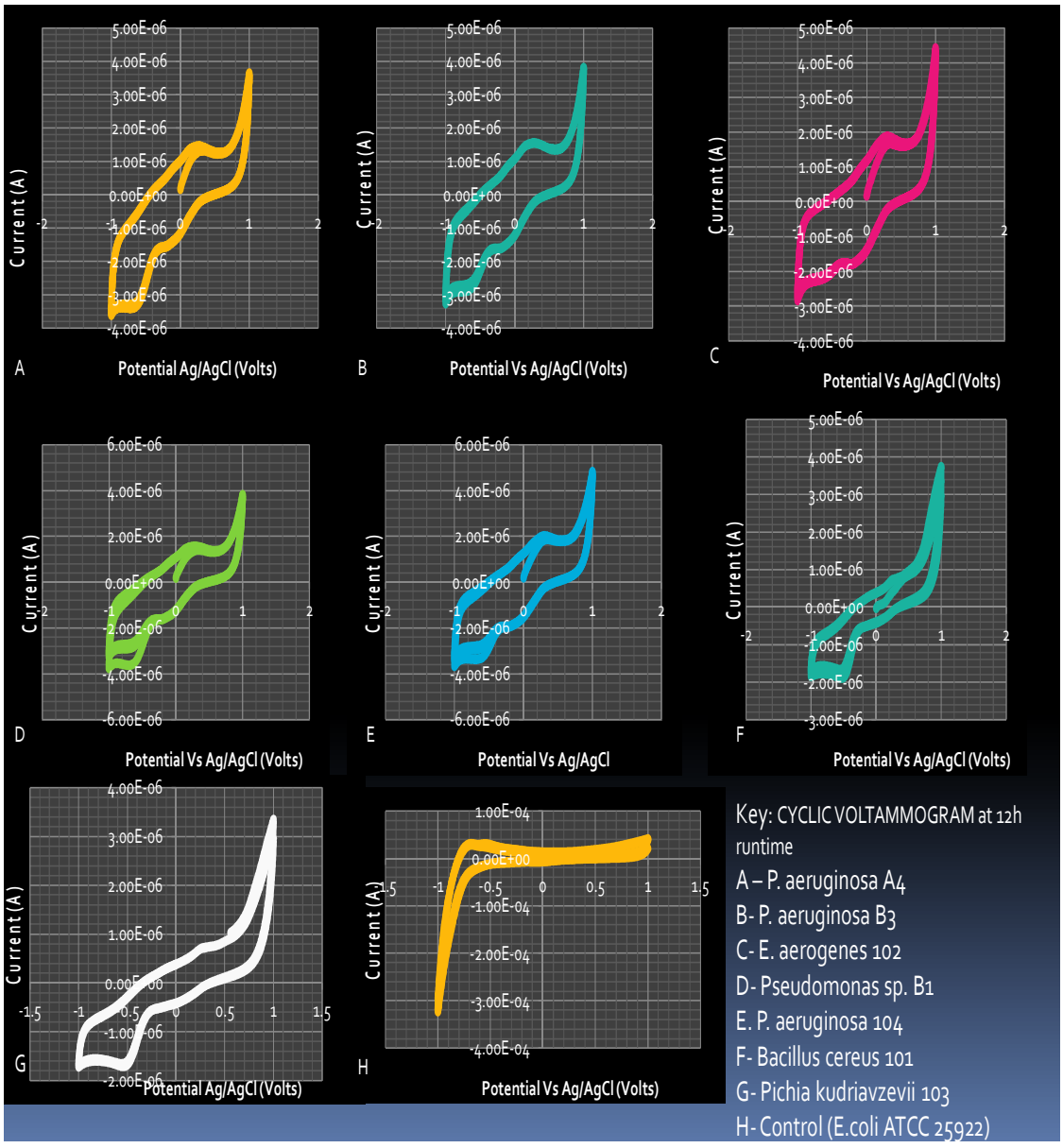

Fig. 3. Cyclic voltammograms of each electrogenic isolate and a control non-electrogenic strain of *E. coli* ATCC 25922

Table 3. Maximum values of bioelectricity generated in MER system for test electrogenic isolates

Single culture MER set-up	^Max. Voltage generated (mV)		^Max. Current (mA/m^2)	^Max. Power (mW/m^2)	*Coulombic efficiency (%)	*Max. Energy (mJ/m^2)
	OCV	CCV				
P. aeruginosa A4	**563.39±3.42**	**483.31±9.16**	**86.37±14.52**	**52.19±10.18**	9	**252**
P. aeruginosa B3	558.19±8.33	481.15±3.47	73.31±3.51	43.18±6.24	8	249
E. aerogenes 102	554.22±13.18	473.42±14.52	54.37±3.01	33.16±5.31	**10**	240
Pseudomonas sp. B1	550.74±13.06	454.36±12.08	50.13±2.14	21.24±7.23	5	214
P. aeruginosa 104	548.26±12.54	450.19±10.19	31.28±5.18	17.18±8.18	6	200
B. cereus 101	545.85±12.45	436.11±4.57	23.05±5.04	11.54±6.05	7	181
P. kudriavzevii 103	538.15±13.81	419.18±7.04	19.22±9.02	8.01±3.11	5	173

Key:
OCV: Open circuit voltage
CCV: Closed circuit voltage
^Measured values are represented as mean±standard deviation
**Calculated values are represented as single value interpretations of calculations using mean values*
*Highest values are written in **bold***

Based on cyclic voltammetry all test isolates were electrochemically active. The oxidation and reduction curves at different currents clearly implied their electroactivity when compared with the non-electrogenic control *E. coli* ATCC 25922. Sacco et al. [10], Kim et al. [39], Rabaey et al. [40], and Niessen et al. [41], all determined from similar examinations that observable cyclic curves of oxidation reduction as detected in this research connote microbial electrogenicity. The similarity in CV curve patterns for all test *Pseudomonas* inferred a uniform biomechanisms of electrogenicity in all the *Pseudomonas* strains. The same could be said of the electrogenicity of *B. cereus* 101 and *P. kudriavzevii* 103 as detected by their similar CV curve patterns. There was however a difference in the CV plot pattern for *E. aerogenes* 102 from that of other isolates tested thus showing a probable difference in *Enterobacter* electrogenic biomechanisms in comparison with all other test electrogens.

3.3 Bioelectricity Measurements

The bioelectricity measurements carried out showed open circuit voltage values ranging from 538.15 mV to 563.39 mV for all electrogenic isolates tested (Table 3), with *P. aeruginosa* A4 yielding the highest OCV and *P. kudriavzevii* 103 giving the lowest. Maximum current and power density ranged from 19.22 to 86.37 mA and from 8.01 to 52.19 mW respectively. A Coulombic efficiency of 10% was achieved with the *E. aerogenes* 102 MER and was the highest Coulombic efficiency recorded. Both *Pseudomonas* sp. B1 and *P. kudriavzevii* 103 gave the least Coulombic efficiency of 5% from each of their MER systems.

4. CONCLUSION

The experiments conducted in this work have shown that there is a prevalence of electrogenic species in environmental sites around known human habitats. The geologic/environmental uniqueness of each site was not considered, however, it is clear that the microbial diversity of electrogens can be strongly linked with electrogenicity based on specie/strain specificity. All genera identified have been earlier implicated as electrogens by previous research works. However, with respect to species, this is the first report of a *Pichia kudriavzevii* strain as an electrogen. This thus implies that to obtain novel genera and species, it is important to screen more environmentally diverse sites, as well as enhance the selectivity process of extracting high electrogenic strains from those environments. The strains isolated proved to have good bioelectricity generation potentials in single culture experiments. This buttresses the point that single culture bioelectricity generation in MER can be achieved with the aid of defined conditions. Further work shall include physiological studies of the biomechanisms of each isolate's electrogenicity as well as optimisation metabolic enhancement of isolate-specific bioelectricity yield.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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