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Isolation of Exoelectrogenic Bacteria from Palm Oil Mill Effluent and Their Application in Microbial Fuel Cell

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Abstract

The ability of exoelectrogenic bacteria to directly transfer electrons without any mediators to extracellular electron acceptors is vital in microbial fuel cell technology. The current study evaluates the exoelectrogenic potential of bacteria isolated from Palm Oil Mill Effluent (POME) in microbial fuel cell. The POME samples were obtained from Palm Oil mill factory in Iwo, Osun State, Nigeria. The isolates were analysed on Chromogenic (differential) medium for colour change from black to whitish. The isolates were identified phenotypically and molecularly. The potential of the isolates to generate efficient electricity were investigated in a double-chambered Microbial Fuel Cell (MFC). Overall, ten isolates were obtained from POME sample, with only three isolates showing the exoelectrogenic potential by turning the agar colour from black to whitish. The molecular analysis revealed three novel strains as *Bacillus velezensis* strain AAS001 (OQ690764), *Bacillus amyloliquefaciens* strain AAS002 (OQ690765) and *Priestia aryabhattai* strain AAS003 (OQ690766). Strain AAS003 showed the highest voltage potential of 1407mV compared to strain AAS001 with 229mV and strain AAS002 with 191mV. Similarly, the power and current densities (345 mW/m^2 and 437 mA/m^2 respectively) recorded by strain AAS003 were far superior to that of strain AAS001 (10 mW/m^2 and 64 mA/m^2) and strain AAS002 (15 mW/m^2 and 92 mA/m^2). This study suggests that strain AAS003 is an excellent biocatalyst for bioelectricity generation.

Keywords: Exoelectrogens; POME; Bioenergy; Microbial fuel cell; *Priestia aryabhattai*.

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Introduction

The need for alternative energy sources has increased due to the limited availability of fossil fuels. Microorganisms have the potential to create biofuels such as biodiesel, bio-alcohols and biohydrogen. These biofuels are also known as alternative fuels from biomass. Biofuels such as biodiesel (Ibeto et al., 2011), bioalcohols (Asad-ur-Rehman et al., 2008), biohydrogen (Abibu and Karapinar, 2023) have been thoroughly described in previous studies. Microbial fuel cell (MFC) technology has been used to generate electricity from microorganisms in an efficient manner (Sirajudeen et al., 2021a). Microbial fuel cells function by utilizing microorganisms as biocatalysts to convert stored energy from organic substrates into electricity. In contrast to normal fuel cells where chemical oxidants catalyse the fuel, in microbial fuel cells, electrons generated by the fuel travel across the anode to the cathode where an electron acceptor gets reduced, thereby generating an electric current (Hindatu et al., 2017).

To successfully implement the MFC technology on a large-scale application, several improvements is needed in the operational sustainability and the material science aspect of the device to reduce the cost and increase the power output. The electrochemical performance of MFC is mainly determined by numerous factors such as the type of inoculum (biocatalyst), electrode materials and proton exchange membrane (PEM) type (Sirajudeen and Annuar, 2021). An appropriate biocatalyst should be thermal-stable for tropical application, proficient in waste degradation and able to release efficient electrons for complete redox reaction (Logan, 2008).

Certain anaerobic bacteria, called exoelectrogens, can produce electricity when given an electron acceptor or some mediators to help with electron transfer. Exoelectrogens such as *Shewanella oneidensis, Geobacter sulfurreducens* are involved in the synthesis of appendages capable of transferring electrical current called nanowires (Logan et al., 2019). These bacteria are electrochemically active and are typically metal-reducing anaerobic bacteria that use metal ions for electron transfer. Unlike other anaerobes that can only transfer electrons to soluble compounds, exoelectrogens can transport electrons outside of the cell (Sun et al., 2014). The anaerobic nature of palm oil mill effluent (POME) suggests the availability of exoelectrogenic bacteria in such environment. Hence, the aim of the current research is to isolate exoelectrogens from POME and their subsequent utilisation in MFC for electricity generation. The search for an efficient electrogens will tackle a vital part of MFC technology as an alternative for renewable energy source.

Materials and Methods

Sample collection

Palm Oil Mill Effluent sample was collected from Palm Oil mill factory in Iwo, Osun State (7.6536763°N, 4.1774621°E). From three different points, samples were taken at a depth of 10-20cm. The POME sample was stored in a sterile container and labelled appropriately. The sample was then transported to Fola Lasisi laboratory at the Department of Biological Sciences, College of Natural and Applied Sciences, Crescent University, Abeokuta, Nigeria for further analysis.

Preparation of media

Chromogenic agar was used in the current study as reported by Nazeer and Fernando (2022). The medium consists of 0.2g of glucose, 0.046g of NH_4Cl, 0.01g of yeast extract, 0.05g of peptone, 0.505g of K_2HPO_4, 0.284g of KH_2PO_4 ,0.0025g of ascorbic acid, and 1.5g of agar-agar. All constituents were dissolved in 100 mL of sterilized distilled water. After autoclaving the medium, 0.15g of MnO_2 was added asceptically. All the chemical reagents used for the preparation of culture media were of analytical grade.

Isolation of exoelectrogenic bacteria

Using the pour plate method, 1 mL each from 10^{-2}, 10^{-4} and 10^{-6} dilution factors were inoculated in the chromogenic agar, which was then incubated for 24 hours at 37^{o}C. Pure cultures were put on nutrient agar slant for further analysis (Onajobi et al., 2019).

Phenotypic Identification of the Isolates

The isolated bacteria were identified by observing their colonial characteristics (colour, shape and size), morphological characteristics (Gram staining) and biochemical tests (Adeyemi et al., 2016; Sirajudeen et al., 2024).

Molecular characterisation of the isolates

The three exoelectrogenic bacteria isolated from POME sample (the isolates that turned the chromogenic agar from black to whitish) were identified using molecular methods at Inqaba Biotech West Africa, its subsidiary at Moniya, Ibadan, Oyo state, Nigeria to ascertain the identity of the isolates. DNA extraction, PCR amplification of *16S rRNA*, and sequencing were carried out using methods reported by Wani et al., (2022).

Microbial Fuel Cell Set-up and Operation

The microbial fuel cell was set up by wielding two screw capped plastic containers which serves as the anode (anaerobic) and cathode (aerobic) chamber. The two chambers were bridged with a screw pipe and Nafion 117 membrane which serves as a proton exchange membrane (PEM). The PEM is a semi-permeable membrane which serves as a physical barrier that separates the anode and cathode compartments while allowing protons to flow into the cathode, so as to produce electrical current. Both the anode and cathode electrodes are made up of 5 cm × 5 cm surface area stainless steel mesh (SSM).

The anolyte composition in 1L distilled water consists of 1g of glucose, 10.7g of K_2HPO_4, 5.3g of KH_2PO_4, 0.04g of $CaCl_2.2H_20$, 1g of NaCl, 0.3g of $MgSO_4.7H_20$, 0.2g of $NaHCO_3$, 0.3g of NH_4Cl, 0.1% trace element. The mixture was autoclaved at 121°C for 15 minutes. The total anode volume was 500 mL consisting of 50 mL inoculum (10%) and 450 mL anolyte. The cathode solution was prepared measuring 8.232g of $K_3Fe(CN)_6$ in 500 mL of sterile distilled water. The electrodes were externally connected with copper wire across 1000 ohms external resistor. The voltage generated by each MFC was monitored using a voltmeter for a complete growth cycle. The polarization data was recorded at maximum and stable voltage during MFC operation using external resistors ranging from 55 – 10,000 ohms (Sirajudeen et al., 2021b; Yusuf et al., 2019).

Results

Isolation and characterisation of bacteria

Table 1 shows the cultural and morphological characteristics of the bacteria isolated from the POME samples. A total of ten (10) bacteria were isolated from the POME sample at 37°C under aseptic conditions. Three of the isolates were able to utilize the MnO_2 in the medium, thereby turning its colour from black to whitish. Culturally, some isolates appeared creamy, serrated, smooth and flat, entire and circular, flat and yellow. The morphological studies of the cell showed four (4) rods, four (4) long rods and two (2) cocci. Out of the ten (10) bacteria isolates, three (3) appeared in pairs, four (4) were in chains and three (3) were in clusters.

Table 1: Cultural and Morphological Characteristics of Bacteria Isolated from POME

S/N	Isolate Code	Cultural characteristics	Cell Shape	Arrangement
1	A1	Creamy, undulate and raised	Rods	Pairs
2	A2	Yellow, serrated and flat	Long rods	Pairs
3	A3	White, raised and smooth	Long rods	Pairs
4	A4	Cream, serrated and flat	Rods	Chains
5	A5	Yellow, circular and entire	Coccus	Clusters
6	A6	White, circular and flat	Long rods	Chains
7	A7	Cream, smooth and flat	Rods	Clusters
8	A8	Yellow, serrated and raised	Coccus	Clusters
9	A9	Creamy, smooth and raised	Rods	Chains
10	A10	Yellow, entire and raised	Long rods	Chains

The physiological and biochemical characteristics of bacteria isolated from POME samples are presented in Table 2. Physiologically, all the isolates showed growth at 37°C. Biochemically, all the ten (10) isolates were catalase and urease positive. Eight (8) isolates were motile, seven (7) isolates were Gram positive, five (5) isolates were citrate positive.

The ten (10) bacteria isolated were identified according to *Bergey's Manual of Descriptive Bacteriology*. The bacteria isolates identified were *Pseudomonas spp., Enterococcus spp., Bacillus spp., Priestia sp.,* and *Micrococcus sp.*

Table 2: Biochemical characteristics of the Bacterial Isolates

S/N	Isolate Code	Motility	Gram's reaction	Citrate	Urease	Catalase	Probable Identity
1	A1	+	-	+	+	+	*Pseudomonas sp.*
2	A2	+	+	+	+	+	*Enterococcus sp.*
3	A3	+	-	+	+	+	*Pseudomonas sp.*
4	A4	+	+	-	+	+	*Bacillus sp.*
5	A5	+	+	-	+	+	*Bacillus sp.*
6	A6	+	+	+	+	+	*Priestia sp.*
7	A7	+	-	+	+	+	*Pseudomonas sp.*
8	A8	+	-	+	+	+	*Pseudomonas sp.*
9	A9	+	+	+	+	+	*Micrococcus sp.*
10	A10	+	+	+	+	+	*Enterococcus sp.*

Key:

+ = positive

- = negative

Identification of bacterial isolates by 16S rDNA gene sequencing

Isolates A4, A5 and A6 were selected for molecular analysis as they are the only exoelectrogens (they utilized MnO_2 by turning the colour of the agar from black to whitish) among the ten (10) isolates. The *16s rRNA* gene sequence of the three isolates were subjected to blast online tool in the database of NCBI

(National Centre for Biotechnology Information) GenBank and it revealed the isolates A4, A5 and A6 to be new strains of *Bacillus velezensis, Bacillus amyloliquefaciens,* and *Priestia aryabhattai* respectively. The new strains of *Bacillus velezensis* (OQ690764)*, Bacillus amyloliquefaciens* (OQ690765) and *Priestia aryabhattai* (OQ690766) were named AAS001, AAS002 and AAS003 respectively

Construction of phylogenetic tree

Figure 1 illustrates how the phylogenetic tree was created using MEGA 11. Genetic relationships exist between strains AAS001, AAS002, and AAS003. Seven nucleotide sequences were used in this investigation. Gaps and missing data were removed from every spot. In the final dataset, there were 549 locations altogether (Tamura et al., 2021).

Microbial Fuel Cell Operation

Strains AAS001, AAS002 and AAS003 were inoculated into the anode chamber of microbial fuel cells for electricity generation. Strains AAS001, AAS002 and AAS003 lasted for a span of nine (9), five (5), and twelve (12) days respectively (Figure 2). The variation in numbers of days is due to the length of the microbial fuel cell cycle, each isolates defers. Amongst the three isolates, strain AAS003, which is *Priestia aryabhattai* has the highest voltage potential of 1407mV, followed by *Bacillus velezensis* strain AAS001 with voltage potential of 229mV, with *Bacillus amyloliquefaciens* strain AAS002 showing the least voltage potential of 191mV (Figure 2).

The power density (PD) and current density (CD) are shown in Figure 3 and Figure 4 respectively. The lowest PD (10 mW/m^2) was recorded by strain AAS001 at a CD of 64 mA/m^2. Strain AAS002 recorded slightly higher PD of 15 mW/m^2 at a CD of 92 mA/m^2, while *Priestia aryabhattai* strain AAS003 recorded the highest PD (345 mW/m^2) and CD (437 mA/m^2).

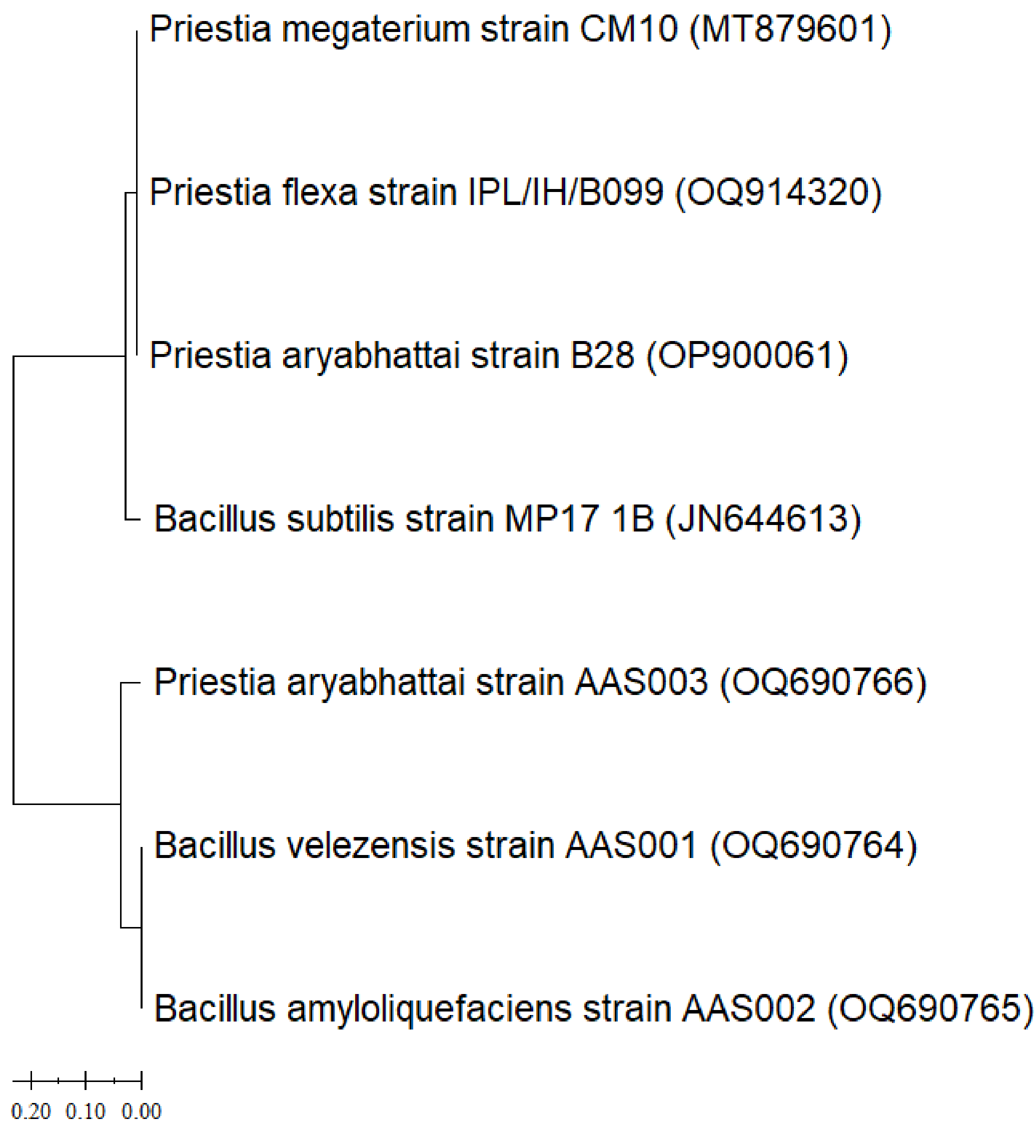

Fig 1: Neighbour-joining phylogenetic tree of the bacterial strains together with NCBI strains

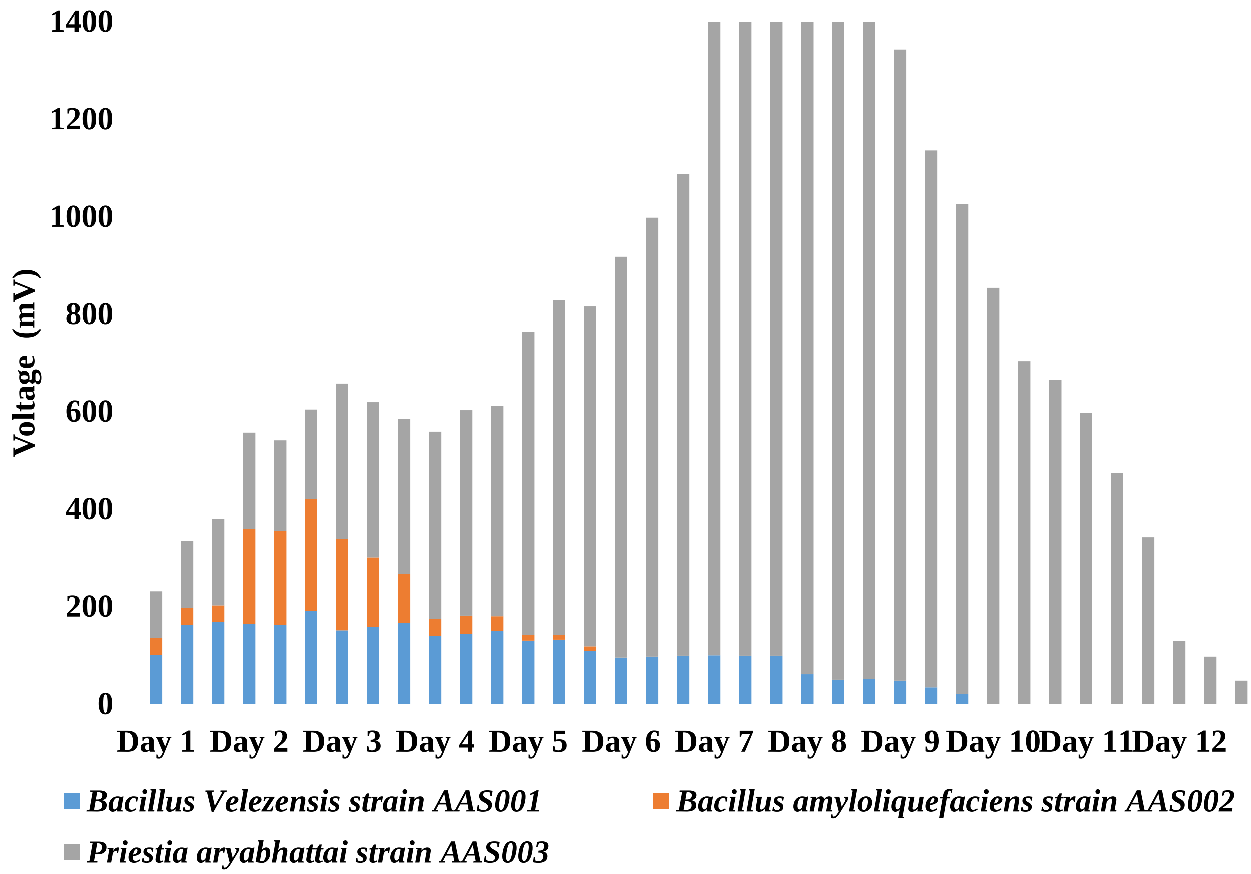

Fig 2: Voltage-time curve of the exoelectrogens during MFC operation.

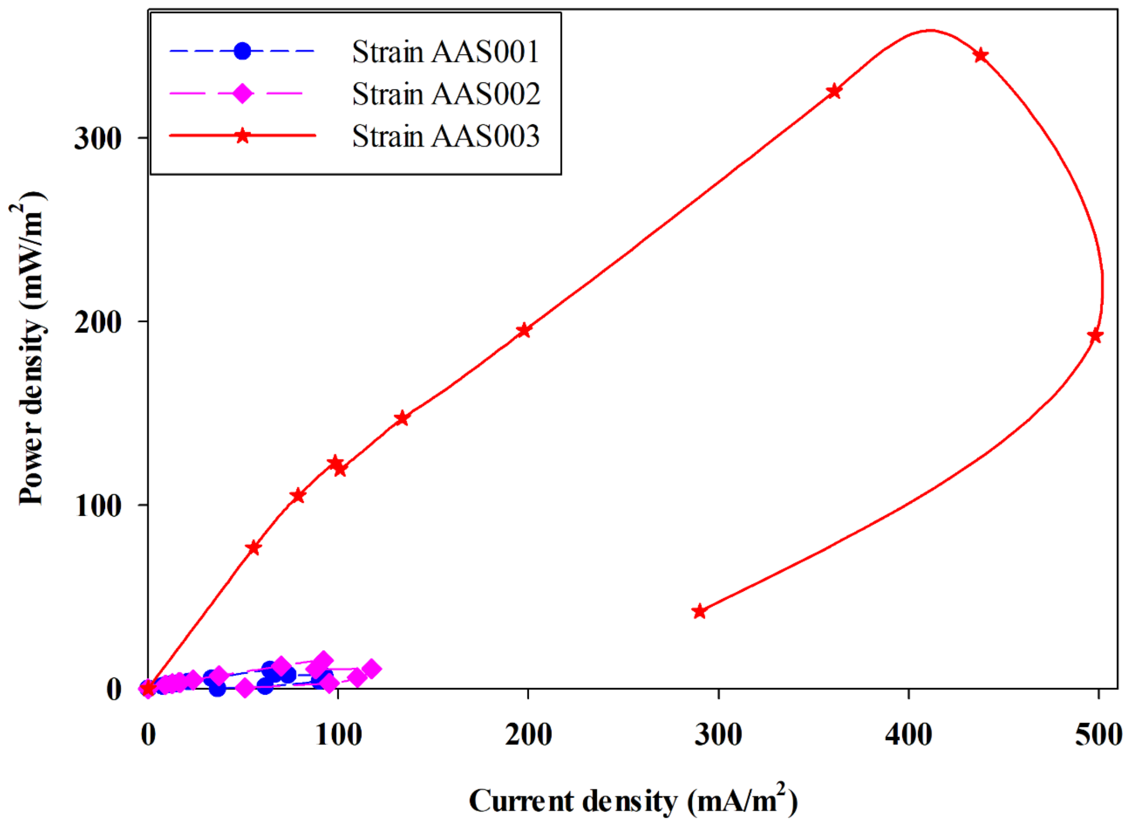

Fig 3: The power density curves of the three strains during MFC operation

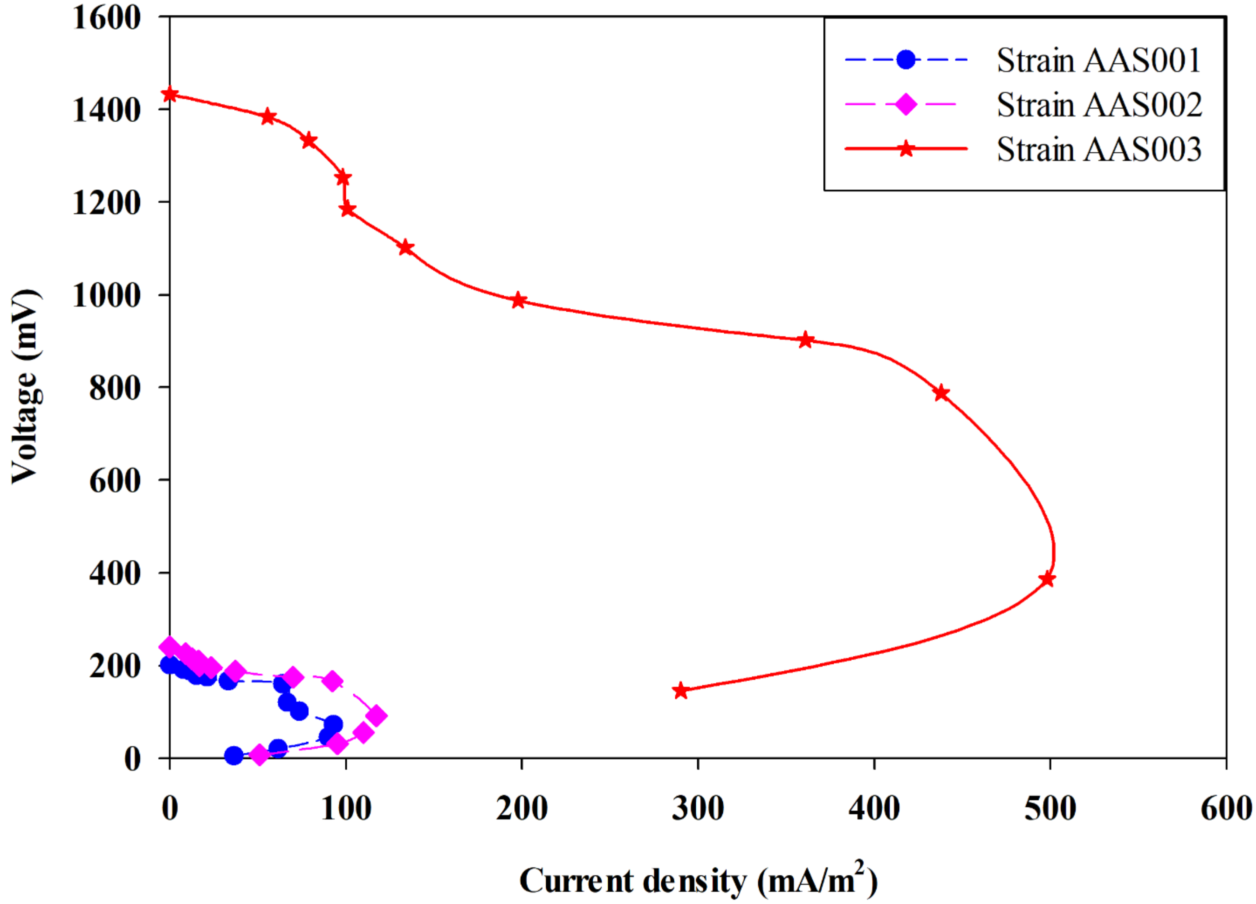

Fig 4: The current density curves of the three strains during MFC operation

Discussion

There are various microorganisms that have been discovered to have the ability to transfer electrons to an anode using energy from metabolic processes. These microorganisms include Proteobacteria, Cytophagales, Firmicutes, Acidobacteria, and yeasts, which can be found in sources such as marine sediment, soil, fresh water sediment, wastewater, and activated sludge, such as POME (Kadier et al., 2023; Logan et al., 2019). This ability to produce electricity has been studied by researchers and has potential applications in various fields (Pisciotta et al., 2012). *Geobacter sulferreducens, Rhodoferax ferrireducens, Shewanella putrefaciens, E. coli* etc are some of these microbes, that has this ability. Bacteria strains that have exoelectrogenic potential have been reportedly isolated from POME samples (Rahimnejad 2009). They are usually Gram-positive bacteria, with a large belonging to the genus *Bacillus.* From this research, two different species of *Bacillus* and one of *Priestia* (previously classified as *Bacillus*) were isolated from POME, this is in accordance with the work of Félicité et al., (2021), who isolated *Bacillus* and *Acinetobacter* from POME samples. It also correlates with a work done by Jeremiah et al., (2018) who also isolated *Bacillus subtilis* and *Bacillus aureus* from POME samples for wastewater treatment in Malaysia.

The three exoelectrogenic bacterial isolated from this study were biochemically identified to be *Bacillus sp*. (1), *Bacillus sp.* (2) and *Priestia sp.* The 16S ribosomal RNA gene sequencing of the isolates revealed them to be *Bacillus velezensis* strain AAS001 with assession number OQ690764, *Bacillus amyloliquefaciens* strain AAS002 with assession number OQ690765 and *Priestia aryabhattai* strain AAS003 with assession number OQ690766. The phylogenetic tree of the isolates explains the evolutionary relationship of the isolates as compared to other isolated strains in the NCBI Genbank, that is, it shows the closeness in their genetic makeup. The three strains from the current study were more related that other *Bacillus* and *Priestia species* found in the GenBank, indicating the genetic diversity and relationship of the three strains to one another. Similar result was reported by Bala et al., (2015).

The ability of an inoculum to generate efficient and stable electricity is the hallmark of MFC technology. *Priestia aryabhattai* isolated from the current study generated superior maximum and stable voltage of 1407 mV between day 7 and day 9 of MFC operation. *Bacillus velezensis* and *Bacillus amyloliquefaciens* generated significantly lesser maximum voltage (229 mV and 191 mV respectively). Similarly, the power density produced by *Priestia aryabhattai* during MFC operation was 35 and 23 times higher than that generated by *Bacillus velezensis* and *Bacillus amyloliquefaciens* respectively. The maximum voltage generated by *Priestia aryabhattai* was superior to previous studies (Hindatu et al., 2017; Salisu et al., 2023; Yusuf et al., 2019). It should be noted that the current study was carried out under no modifications, and further modifications of the MFC operation parameters is expected to generate efficient and stable power output. The result is in accordance with the work of Islam (2017). Furthermore, *Priestia aryabhattai* has never been reported as a biocatalyst in MFC. This novel study is the first to report the use of the bacterium in MFC technology.

Conclusion

The electrogenic bacteria isolated in this study may offer several advantages for the development of efficient microbial fuel cells. These bacteria, especially *Priestia aryabhattai* have the potential to enhance power generation in MFC, facilitate the degradation of organic pollutants, and contribute to the overall sustainability of palm oil mill operations. Future research will focus on the utilization of palm oil mill effluent as a feedstock for microbial fuel cell using these isolates, which will address both environmental concerns associated with effluent discharge and the growing demand for renewable energy sources.

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