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A STUDY ON BIOELECTRICITY GENERATION FROM THE SEA WATER USING MICROBIAL FUEL CELL

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ABSTRACT

Microbial Fuel Cells (MFCs) use bacteria as biocatalyst to convert biodegradable substrates into electricity. The normal sea water generated maximum Open Circuit Voltage (OCV) of 4.5mV while sterile sea water generated only 0.9mV OCV in the H-shaped MFC. On optimization of MFC setup, 100% sea water and a salt bridge (5cm × 2cm) containing a mixture of 10% sodium chloride and 5% agar, and electrodes of 32.20cm² surface area was found ideal. Furthermore, two electrogenic bacteria were isolated from the sea water and individually studied for their electrogenicity. The isolate SWA1 was selected as electrogenic bacteria, as it generated 754.7mV OCV at 37°C, pH 7.0 with LB medium as anolyte against vinegar as catholyte after 24h inoculation in MFC. Based on phenotypic characteristics and 16S rDNA sequencing, isolate SWA1 was identified as *Pseudoalteromonas* sp.

Keywords: Bioelectricity, Electrogenic bacteria, Microbial fuel cell, Open circuit voltage, Sea water

INTRODUCTION

World energy demand is expected to raise from 421 quadrillion British Thermal Units (BTUs) in 2003 to 563 quadrillion BTUs in 2015 to 722 quadrillion BTUs in 2030 [1]. Energy production from renewable feed stocks holds great potential to meet these needs in a sustainable and environmentally sound manner and to reduce dependence on fossil fuels. Recently, microbial fuel cells (MFCs) have drawn increasing worldwide attention in directly generating electricity from organic matters [2, 3]. Microbial fuel cells (MFCs) are Bio-Electrochemical Systems (BESs) [4], which utilize bacteria to oxidize organic matter and transfer electrons to the anode, where they flow to the cathode and react with protons and oxygen to form water [5]. Bacteria which are useful in microbial fuel cell operation have the ability to transfer electrons to an electrode (anode), as a terminal electron acceptor are classified as Electrochemically Active Bacteria (EAB) or Electrogenic bacteria [6]. Conventional methods

of isolating exoelectrogenic microorganisms are based primarily on identifying microorganisms that can respire using soluble or insoluble metal oxides in agar plates [7, 8]. However, not all dissimilatory metal oxide reducing bacteria are capable of producing electricity in an MFC, and not all bacteria that produce current in an MFC can grow using metal oxides [9,10]. Therefore, these methods may miss important electrochemically active strains of microorganisms.

Until date, several reports have been documented on the electricity generation from the different natural waste matter like land fill, sea sediments and waste water; however a very less reports are available where normal sea water samples containing bacteria while organic and inorganic compounds in it used as fuel to monitor its potential for the electricity generation using two chambers MFC. The present study done focus on the determination of electric potential of normal sea water to generate the electricity in fabricated

two chamber MFC followed by screening and identification of potent electrogenic bacteria present in it.

METHODS AND MATERIALS

Collection of samples

The sea water sample was collected in sterile containers from the Kanyakumari, Tamil Nadu, India whereas a liter of sewage wastewater from Meerut, Uttar Pradesh, India was also collected, carry and stored at 4°C until employed in the experiment.

Initially, the studies were carried with sea sediment samples as a sole anolyte against vinegar, to note the bioelectrical contribution of the microbial flora present in it.

MFC fabrication and operation

The H-shaped MFCs were fabricated with two polycarbonate bottles (500 mL) as chambers and a PVC pipe (5cm × 1cm) for preparing a salt bridge. The salt bridges were prepared by filling boiled sodium chloride (10%) solution containing 5% agar. The salt bridges were fixed to the bottles with the aid of epoxy adhesive (M-Seal, Pidilite Industries Ltd, Maharashtra, India). The sea water was used as anolyte without any pretreatment and vinegar was used as a catholyte in the MFC setups. The two graphite pencils (0.25cm × 10cm, Apollo pencil manufacturers, Mumbai, India) with surface area of 16.10cm² were used as electrodes. To aid initial wetting, the electrodes were boiled in deionized water and soaked overnight in 1M HCl solution followed by thorough rinsing with deionized water [11]. The electrodes were inserted into respective chambers while circuit connections were set with the copper wires fixed into the drilled holes of the electrodes and sealed with epoxy resin to avoid corrosion of copper wire [12]. The fabricated MFCs were sterilized with Ethanol (70% v/v) and irradiated with UV for 15 min followed by electrolytes addition up to the brim of the respective chambers. After 24h, OCV from the MFCs was recorded at room temperature without any external resistance until 40min with an

interval of 2min using the multidigital meter (UNI-DT830D, Uni-Trend Group Ltd., Kowloon, Hong Kong).

Optimization of MFC operation

Since, it is necessary that MFCs should be optimized in terms of reactor configuration and physiochemical parameters, MFCs were operated using different concentrations of sea water (100, 75, 50, and 25%), 10% concentration of different salts; sodium chloride(NaCl), potassium chloride(KCl), ammonium nitrate(NH₄NO₃), ammonium chloride(NH₄Cl) and mixed salts (2.5% each salt), different concentrations of optimized salt (5, 10,15 and 20 %), different lengths of salt bridge (1.5, 3 and 5cm), various radius of salt bridge (1cm and 2cm),different surface area of anode (16.1cm² and 32.2cm²), exogenous mediators (phenol red and neutral red) while potential difference was monitored in terms of OCV. During the optimization experiment, the optimized parameter was employed in the optimization study of another parameter.

Sea water verses sewage water

The optimally fabricated MFC setup was monitored for the OCV generation with the sea sediments as anolyte against 500mL of sewage water as catholyte in the respective chambers of the MFC as described earlier section.

Isolation of bacteria from sea sediment

The sea water sample was serially diluted with saline water (0.85% w/v NaCl) and the 10⁻⁵ dilution of the sample (0.1mL) was then spread on the LB agar plate (Tryptone 10.0g, NaCl 10.0g, Agar 20.0g and Yeast extract 5.0g in 1000mL distilled water) further incubated for 24h at 35°C. Morphologically distinct bacterial colonies were purified and further studied for their Gram staining properties [13].

Physiochemical growth optimization of bacteria

The LB broth prepared were inoculated with the 24 h old sea water isolates culture (1 %, v/v) separately were studied for the bacterial growth at different pH (4, 5, 6, 7, 8, and 9) and temperature

(27, 37, 47 and 57°C) by measuring absorption at 660 nm, against the sterile LB broth as blank [14].

Electricity generation and identification of potent electrogenic bacteria Using optimized growth and MFC parameters, the isolates were studied for their electrogenicity with 500mL LB broth culture (24h old) as anolyte against 500mL vinegar as catholyte in terms of OCV. The potential isolate screen out was then outsourced to Bioserve Biotechnologies Pvt. Ltd, Hyderabad, India, for molecular identification by 16S rDNA technique. The 16S rDNA sequence obtained was initially analyzed at NCBI server (<http://www.ncbi.nlm.nih.gov/>) using BLAST (Blastn) tool and corresponding sequences downloaded were further used for phylogenetic analyses using MEGA version 4 [15].

RESULTS

Analysis of electricity from sea water

In order to check the presence of electrogenic bacteria in the sea water samples, electricity generation monitored from the normal sea water samples and sterilized sea water was observed against vinegar (Fig.1). The maximum OCV of 4.5mV was recorded at 16min from the normal sea water while highest OCV profile of 0.9mV at 2min was monitored from the sterilized sea water. This deduced that the normal sea water samples possessed the electrogenic bacteria which contributed into the electricity production.

Analysis of MFC parameters

Concentrations of sea water The sea water sample was diluted with deionized water to various concentrations served as anolyte in the MFCs where OCV was monitored (Fig.2). It was observed that maximum OCV of 4.7mV at 18min was recorded from the MFC containing 100% sea water.

Effect of different salts and concentrations of ideal salt Among the different salts studied in the salt bridge having 10% concentration, the maximum OCV of 4.8mV was recorded by the salt bridge containing NaCl (Fig.3) at 18min with

continuous and steady elevation in the electric potential over time in comparison to other salts. Hence, the NaCl was opted as the suitable salt which was further analyzed for its effective concentration for better OCV.

The concentration of NaCl was further varied and observations revealed that 10% could yield a maximum OCV of 4.9mV at 19min with consistent increment over time (Fig.4).

Dimensions of salt bridge (Length and radius)

Based on optimized NaCl concentration, various lengths of the salt bridge were monitored for the MFC. Fig.5 illustrates highest OCV generation of 4.6mV from the 5cm long salt bridge with a uniform increment over time.

The salt bridges having 5cm length were varied in radius and used in the MFC setups. The OCV profile (Fig.6) reveals that salt bridge 2cm radius could generate more OCV of 5.5mV at 17 min in comparison to that of 1cm radius salt bridge.

Anode surface area

The effect of anodic surface area of 16.10cm² and 32.2cm² were used to check its effect on the OCV from MFC setup. The data obtained revealed that maximum OCV of 5.9mV was recorded with 32.2cm² anodic surface area at 16min (Fig.7). The results concluded that anode with 32.2cm² surface area was ideal for the MFC configuration.

Analysis of exogenous mediator

The electricity generations in terms of OCV were recorded with phenol red and neutral red amended as electron shuttles in the anodic chamber containing 100% sea water as anolyte. The maximum OCV of 1.5mV and 1.9mV were monitored for the phenol red and neutral red at 2min, respectively followed by sudden decrease in the potential (Fig.8).

Analysis of electricity generation from sea water against sewage water The fabricated MFC setup was operated with all the optimized parameters studied and production of electricity was recorded after 24h of inoculation in terms of OCV from MFC with 100 % sea water as anolyte against 500mL untreated sewage water as

catholyte. It was observed that MFC with sewage water as catholyte could produce (Fig.9) maximum OCV of 3.3mV at 13min but fluctuations in the reading was recorded.

Study on sea water bacterial isolates

The two morphologically distinct bacterial colonies isolated were named as SWA01 and SWA1. Gram staining study revealed that the sea water isolate SWA01 was gram-negative cocci and SWA1 was gram-negative rods in nature. The pH 7 and temperature 37°C were recorded as the optimized growth requirements for both bacterial isolate.

Analysis of electricity generation from the sea sediment bacterial isolates

The isolates were independently studied for their potential to produce bioelectricity in the MFCs. Fig.10 represents the OCV generation from the 24h old bacterial isolates cultures recorded for 40 min at the 2 min intervals, inoculated as anolyte in the anodic chambers. The MFC containing isolate SWA1 showed the maximum OCV of 754.7mV at 40min, with significant steady increase over time in comparison to SWA01 in the MFCs.

Identification of isolates SWA1 The bacterial colonies of the isolate SWA1 observed were smooth, circular, light red in color, 1mm in diameter. Furthermore, sequence analysis of the 16S rDNA showed 100% similarity with the *Pseudoalteromonas lipolytica* strain K-W45 (JQ79909). Based on the phenotypic characteristics and phylogenetic analysis using 16S rDNA, isolate SWA1 was identified as *Pseudoalteromonas* sp. The Fig.11 shows the phylogenetic relationship between the isolate SWA1 with the *Pseudoalteromonas* sp. The 16S rDNA sequences for the isolate SWA1 have been deposited at Gene Bank in NCBI with accession number JX105433.

DISCUSSION

The present study concluded that the sea water harbor electrogenic bacteria which lead to the

production of bioelectricity in the fabricated MFCs. The 100% sea water produced more OCV must be because of less number of bacteria in the diluted concentration of sea water. This analysis of different salts for the Salt bridge recommended the use of 10% NaCl concentration as optimized salt in the salt bridge to facilitate easy ion flux. The sodium chloride in the salt bridge yielded good OCV might be because of its good electrolytic property [16]. While 5cm length of salt bridge proved better with 2cm diameter might be because of less density of agar in longer length and easy flow ions through wider diameter. In the fabricated MFC, results concluded that use of anode with 32.2cm² surface area showed more OVC, must be because of more space for the bacteria to liberate the electron. Also, both the exogenous mediators used showed decrease in the OCV. It was reported that most of the exogenous mediators are toxic for the microbes [2]. Electricity generation from the sea water and sewage could not produce good yield might be effect of ions present in both the electrolytes. The bioelectricity generation from the sea water bacterial isolates results indicated that isolate SWA1 contributed more effectively into overall voltage generation from the normal sea water where other strain also generate the potential but fluctuations were observed in the reading obtained during MFC operation. The OCV of 754.7mV was generated by the isolate *Pseudoalteromonas* sp. SWA1 was nearer to that of maximum OCV of 800mV [16] reported until now. This implies that the bacterial isolate SWA1 might have electrogenic properties like C-type cytochromes [17] or conductive nano-wires [18] on its cell membrane to generate electric potential. Also the optimum pH noted for the SWA1 was in accordance with the report in which highest bioelectricity generation observed thus far at pH 7.0 [19].

CONCLUSION

The present study revealed that the potential electrogenic bacteria can be easily procured from

the natural sources like sea water by simple technique of serial dilution and spread plate for operating the MFCs other than sediment or benthic MFCs. The study recommends the further tuning of the technology for the better usage of the potential electrogenic bacterial isolate from the sea water towards the commercial utilization to generation of alternate energy to sustain the demand of future.

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Fig.1: Monitoring of OCV from the normal and sterilized sea sediment from the MFC.

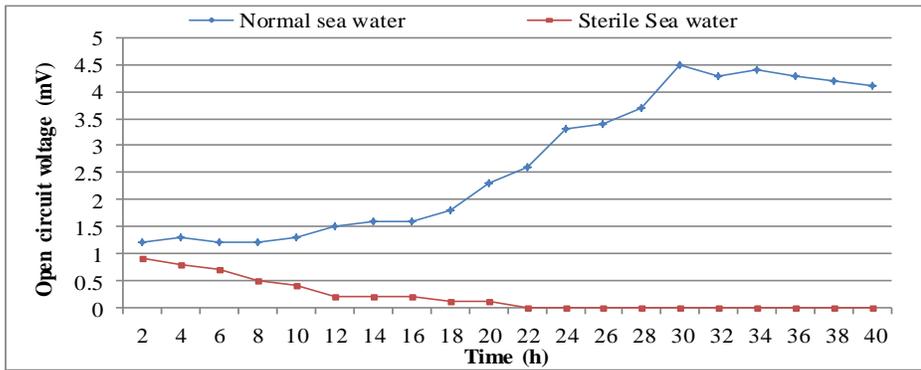


Fig.2: Effect of different concentrations of sea water on the OCV.

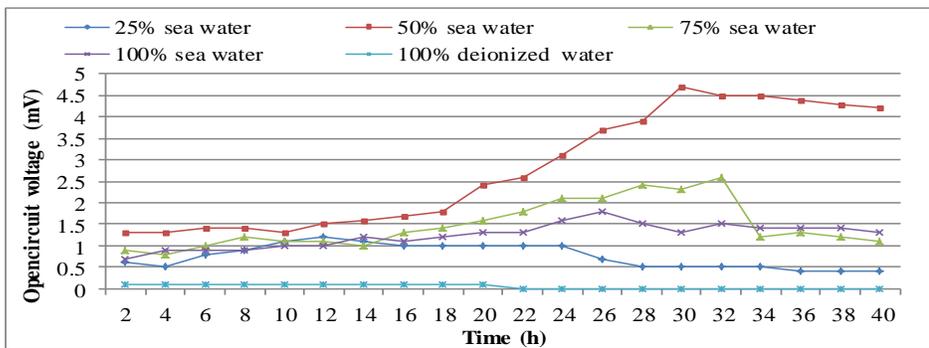


Fig.3: Monitoring of OCV from the MFCs having different salts in the salt bridge.

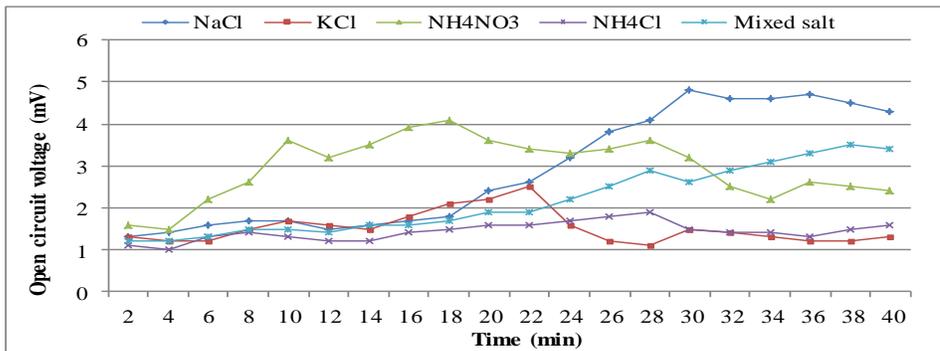


Fig. 4: The OCV recorded for the different concentrations of optimized salt in the salt bridge.

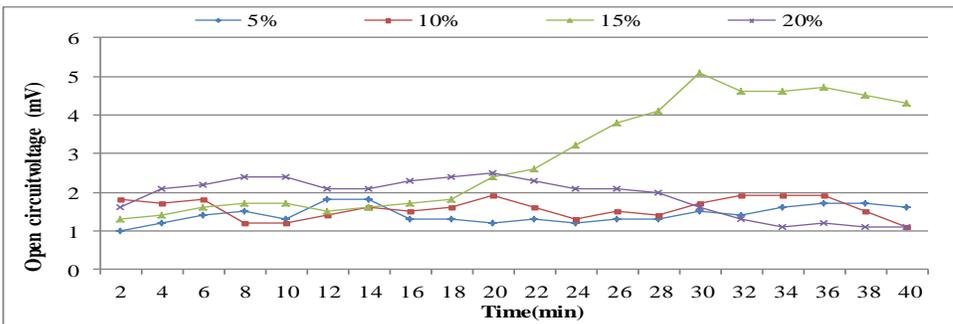


Fig. 5: OCV generated on employing different lengths of the salt bridge in the MFCs.

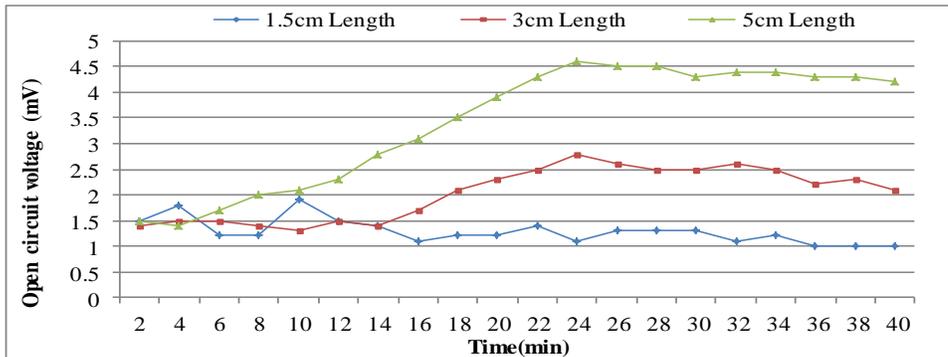


Fig. 6: OCV recorded from the MFCs with the use of different radius of salt bridge in the MFCs.

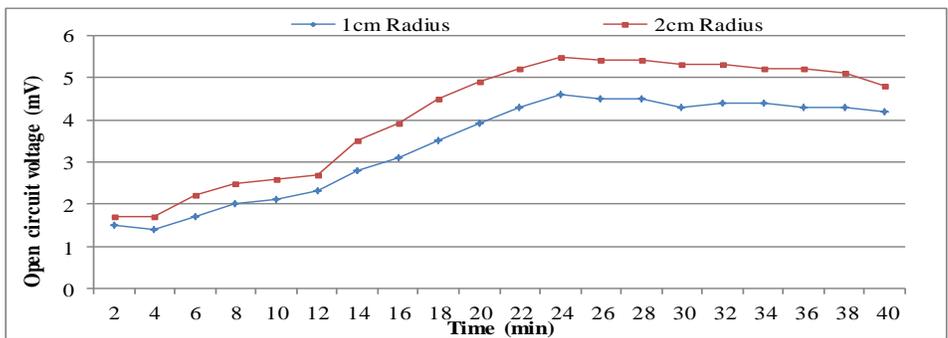


Fig. 7: OCV generation from the MFCs operated with different anode surface area.

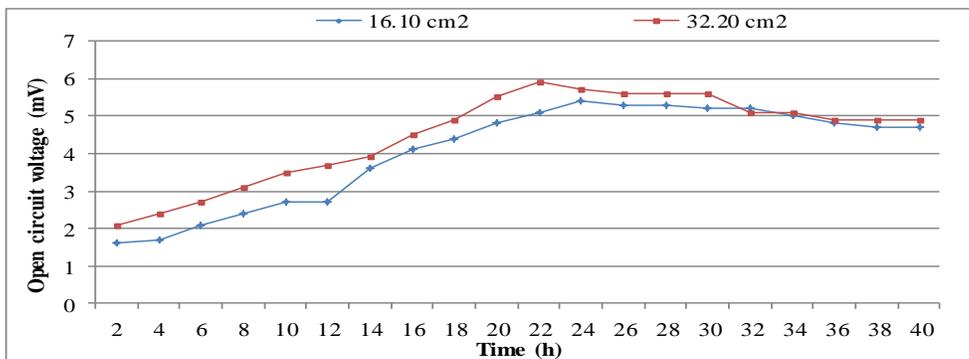


Fig. 8: OCV monitored for the use of exogenous mediators in the MFC.

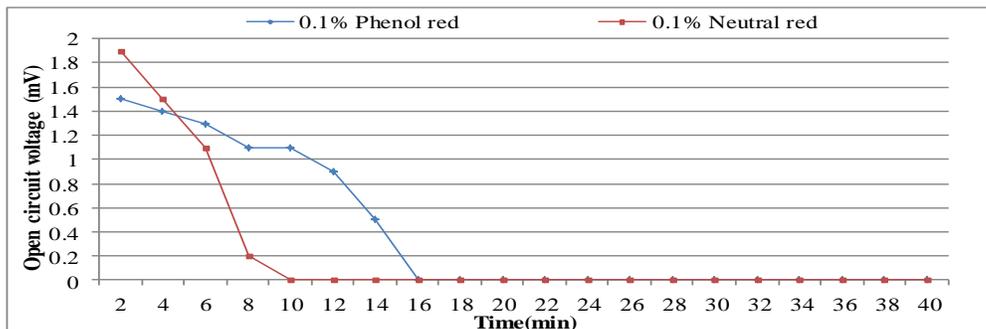


Fig. 9: OCV observed from the MFC containing sea sediment versus Sewage water.

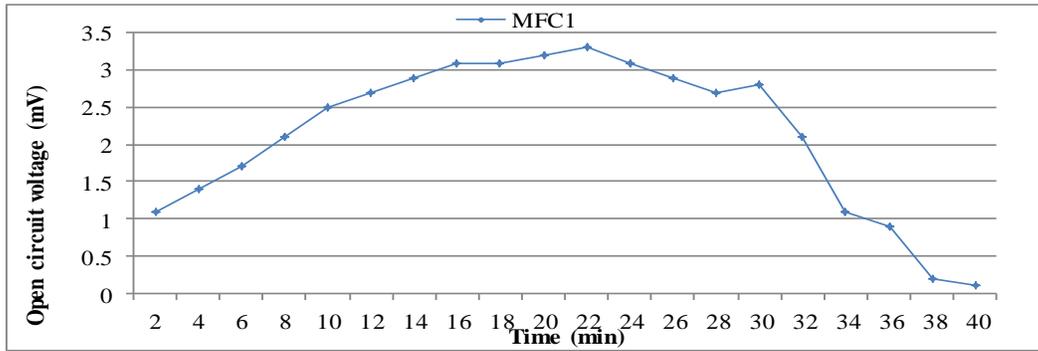


Fig. 10: Analysis of OCV generation from the sea sediment isolates in the optimized MFC.

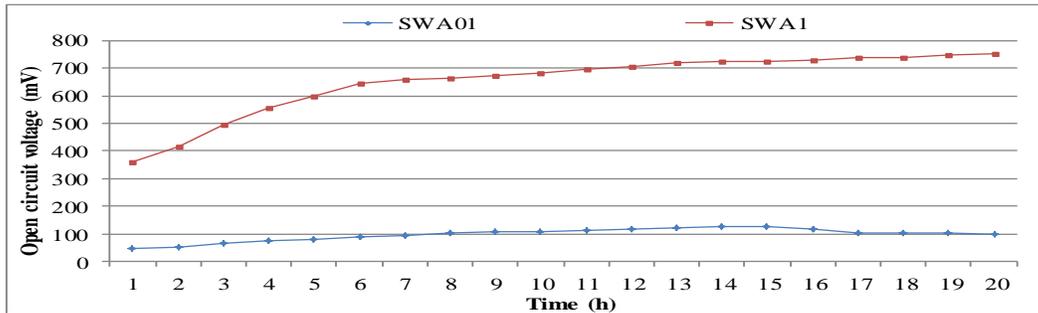


Fig.11: Phylogenetic tree of the isolate SWA1 in relation to *Pseudoalteromonas* species. The tree was constructed using the Neighbor-joining algorithm. Numbers on the tree refer to bootstrap values on 1000 replicates. Nucleotide accession numbers are given in parentheses. The bar indicates a 0.1% estimated difference in nucleotide sequences.

