Applied Environmental Research

Journal homepage : http://www.tci-thaijo.org/index.php/aer

Biosurfactant Production by Rhizospheric Bacteria Isolated from Biochar Amended Soil Using Different Extraction Solvents

Seun O. Adebajo[1,*], Aderonke K. Akintokun[1], Abidemi E. Ojo[1], Dami M. Egbagbe[1], Pius O. Akintokun[2], Lawrence O. Adebajo[3]

[1] Microbiology Department, Federal University of Agriculture, Abeokuta, Nigeria
[2] Department of Plant Physiology and Crop Production, Federal University of Agriculture, Abeokuta, Nigeria
[3] Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria
[*] Corresponding author: Email: adebajoso@funaab.edu.ng

Article History
Submitted: 11 June 2019/ Revision received: 8 August 2019/ Accepted: 15 September 2019/ Published online: 21 November 2019

Abstract

Microbial-derived surface-active compounds (biosurfactants) have attracted attention due to their low toxicity, cost-effectiveness, biodegradable nature and environment compatibility. Due to paucity of knowledge in the production of biosurfactant by microorganisms from other sources such as biochar-amended soil, the present study investigates the potential of rhizospheric bacteria isolated from biochar amended soil of okra plant in the production of biosurfactants using different recovery techniques. Rhizospheric bacteria were screened for biosurfactant production using Haemolytic, Oil spreading, Drop collapse, Methylene blue method, Bacterial adhesion to hydrocarbon and Emulsification activity. The biosurfactant was extracted using different extraction solvents (acid precipitation, ethyl acetate, acetone, dichloromethane and chloroform/methanol). Degradation of hydrocarbon (diesel) was determined spectrophotometrically. A total of twenty-three rhizospheric bacteria were isolated from the soil of *Abelmoschus esculentus* (okra plant). Nine isolates were positive for haemolysis with values between 1.1±0.2 mm by Enterobacter cloaca and 23.0±0.6 mm by *Alcaligenes faecalis*. Two isolates were positive for the drop collapse test. Only one isolate was positive for the methylene blue method. In the oil spreading test, ten isolates were positive and five isolates had the ability to adhere to hydrocarbons. Six isolates exhibited emulsification potential after 24 h, with the highest and lowest (65.9%) and (40.7%) recorded by *Alcaligenes faecalis* and *Citrobacter* sp, respectively. The biosurfactant produced by *Alcaligenes faecalis* using different recovery solvents showed that chloroform and methanol are the best extraction solvents and *Alcaligenes faecalis* was also able to degrade diesel oil over a period of 10 d. Conclusively, *Alcaligenes faecalis* recovered from soil amended sawdust biochar of okra plant is both a potent biosurfactant producer and an agent for remediating hydrocarbon-contaminated soil environments.

Keywords: Biosurfactant; Emulsification; Drop collapse; Hydrocarbon; Rhizospheric bacteria

https://doi.org/10.35762/AER.2019.41.3.6

Introduction

Low molecular weight surface-active compounds widely produced by bacteria, fungi and yeast are known as biosurfactants. Favorable features such as high biodegradability, ecological acceptability and lower toxicity make biosurfactants potentially some of the best alternatives to chemically synthesized surfactants in a variety of applications [1].

Biosurfactants have also been employed to enhance oil production, and especially in tertiary oil recovery, due to their efficiency in lowering interfacial tension in the interfaces between fluids having different polarities, e.g. oil and water [2].

Biosurfactants can be categorized into glycolipids, phospholipids, lipopeptides and polymeric surfactants [3]. Industries such as cosmetics, food, pharmaceutics, agriculture, microbial enhanced oil recovery (MEOR) and bioremediation [4-5]. Also, biosurfactants take part in biofilm formation as well as signaling in plant-microbe interactions. This makes them important especially where they can increase the bioavailability of hydrophobic compounds for plants in the rhizosphere [6-7].

Biochar is a charcoal-like substance produced by pyrolysis of organic material from agricultural and forestry wastes. Biochar material can be used as a soil conditioner, amendment and may also be suitable for use in environmental management [8].

Many studies have been reported on microbially synthesized surfactants derived from hydrocarbon-contaminated sources, but due to paucity of knowledge in the isolation of microorganisms, the present study investigates the potential of rhizospheric bacteria recovered from sawdust biochar soil of okra plant for the production of biosurfactant using different recovery solvents, and also to determine the hydrocarbon degradation activity of potential biosurfactant producing microorganisms.

Materials and methods

1) Preparation of biochar with sawdust biomass

Biomass (sawdust) was sun dried to reach a level of 15%. The biomass was broken up by hand in order to achieve proper heating during pyrolysis and loaded into the inner retort chamber, comprising a 30 gallon drum with perforations in the base. The biomass was heated at a temperature of 400^{O}C. A dense smoky black char produced after 5 h of heating was then removed as the biochar product. Biochar (28 g) was mixed with 5 kg of soil, watered and allowed to settle for 5 d [9]. After 5 d, okra seedlings were sown in the medium.

2) Isolation and characterization of rhizospheric bacteria

One gram of the rhizospheric soil from the okra plant after 9 weeks of planting was collected and serially diluted up to 10-6 with sterile water. Aliquots (0.1 mL) were inoculated into a nutrient agar medium, incubated for 24 h to produce pure cultures. The isolates were identified using biochemical and morphological characterization [1].

3) Screening of rhizospheric microorganisms for biosurfactant production

3.1) Haemolytic activity

Fresh colonies were extracted and smeared on blood agar plates. These plates were incubated at 37°C for 48 h. The plates were then observed for the presence of a clear zone surrounding the colonies, indicating the presence of biosurfactant -producing organisms [10].

3.2) Oil spread technique

This was carried out as described by Ariechi and Guechi [11]. 50 mL of distilled water was added to Petri plate followed by addition of 100 µL of crude oil to the surface of the water. Then, 10 µL of 24 h cell-free culture broth was dropped onto the crude oil surface. The diameter of the clear zone on the oil surface was

measured and compared to 10 µL of distilled water as a negative control.

3.3) Drop collapse test

Mineral oil (100 µL) was inoculated to 96-well microtiter plates and allowed to equilibrate for 1 h. Ten microliter (10 µL) of the 24 h culture supernatant was introduced into the surface of the oil in the well and plates were observed after 1 min. The culture supernatant containing surfactant causes the drop to collapse; if the drop remain intact, this indicates a negative result. Distilled water was used as control treatment [12].

3.4) Bacterial adhesion to hydrocarbon activity

To determine bacterial adhesion to hydrocarbon method using the reported by Goulart et al. [13], bacterial cells were suspended in phosphate buffer salt solution g L^{-1} (K_2HPO_4: 16.9 and KH_2PO_4: 7.3 g L^{-1} with pH 7 to give an optical density of 0.5 at 600 nm. 100 µL of kerosene was added to 2 mL of cell suspension and was vortexed for 2 min in a test tube. The aqueous phase was allowed to separate for 1h, and the optical density of the aqueous phase (A_0) was measured after 10 min. Hydrophobicity was measured as the percentage of cell adherence to hydrocarbon. The degree of hydrophobicity was calculated as $H = 1\text{-}A/A_0 \times 100\%$, where A is the absorbance of the aqueous phase after hydrocarbon was added and A_0 the absorbance of the aqueous phase before hydrocarbon was added.

3.5) Methylene blue method

A mineral salt agar medium supplemented with glucose as a carbon source (2%), cetyltrimethyl ammonium bromide (CTAB: 0.5 mg mL^{-1}) and methylene blue (MB: 0.2 mg mL^{-1}) was prepared. 30 µL of 24 h culture supernatant was loaded into the each well prepared in methylene blue agar plate using a 4 mm cork borer. The plate was then incubated at 37°C for 48 h. A dark blue halo zone around the culture was considered positive for anionic biosurfactant production [14].

3.6) Emulsification activity

This is a confirmatory screening test for biosurfactant production. Emulsification activity was carried out as described by Kebbouche-Gana [15]. 2 mL of crude oil and 2 ml of cell-free medium (supernatant) were measured into a test tube and vortexed at high speed for 2 min. Emulsification activity was calculated at different time intervals using the formula: E (%) = total height of the emulsified layer/total height of the liquid layer × 100.

4) Molecular and phylogenetic analysis of potential isolate

The best biosurfactant producer was selected from the screening tests for molecular characterization. Extraction of the genomic DNA of bacteria isolate, amplification by polymerase chain reaction (PCR) using 16Sr-RNA primer, sequencing of the isolate DNA and DNA sequence were used to identify the isolate. Phylogenetic analysis was carried out on potential biosurfactant producer with seven reference isolates obtained from NCBI database [16-17].

5) Biosurfactant production

5.1) Fermentation medium

For biosurfactant production, a mineral salt medium with the following composition was utilized (g l^{-1}):2.5 g L^{-1} of $NaNO_3$, 0.1 g L^{-1} of KCL, 3.0 g L^{-1} of KH_2PO_4, 7.0 g L^{-1} of K_2HPO_4, 0.01 g L^{-1} of $CaCl_2$, 0.5 g L^{-1} of $MgSO_4 \cdot 7H_2O$) supplemented with 5 ml of trace element solution (0.116 g L^{-1} of $FeSO_4 \cdot 7H_2O$, 0.232 g L^{-1} of H_3BO_3, 0.41 g L^{-1} of $CoCl_2 \cdot 6H_2O$, 0.008 g L^{-1} of $CuSO_4.5H_2O$, 0.008 g L^{-1} of $MnSO_4 \cdot H_2O$, 0.022 g L^{-1} of $[NH_4]_6Mo_7O_{24}$, 0.174 g L^{-1} of $ZnSO_4$) supplemented with 5% pome as substrate, final pH adjusted to

7.0 and autoclaved at 121°C for 15 mins. The medium was allowed to cool, inoculated with 5% of the bacterial broth and incubated at 30°C, 200 rpm for 5 d [2].

5.2) Recovery techniques of biosurfactant

The extraction techniques involve the use of acid precipitation and solvent extraction methods. The fermentation broth sample was centrifuged at 13,000 rpm for 15 min. The obtained supernatant serves as the crude biosurfactant. Crude biosurfactant was treated by acidification to pH 2.0 using 2N HCl and the acidified supernatant was left overnight at 4°C for complete precipitation of the biosurfactant. Precipitated samples were centrifuged at 13,000 rpm for 30 min and the pellets obtained served as the unpurified biosurfactant for acid-precipitated samples. Another crude biosurfactant sample was extracted three times separately with an equal volume of ethyl acetate, acetone, dichloromethane and chloroform/methanol (2:1). The organic solvent was evaporated using a rotary evaporator and the residue obtained served as the unpurified biosurfactant [18].

5.3) Purification of biosurfactant

The modified method of Qiao and Shao [19]; Kim et al. [20] was employed for biosurfactant purification. The biosurfactant produced was allowed to run through open column chromatography packed with Sephadex LH 20 (Sigma Aldrich) as the stationary phase and methanol as the mobile phase. The velocity of flow was 12 s per drop and methanol was the only flow solvent. Eluent from the column was collected in clean glass bottles. At the end of collection, methanol in the glass bottle was allowed to evaporate leaving the fractionated compound. The purified biosurfactant was then weighed.

6) Antimicrobial activity of biosurfactant

Crude supernatant was used to assay for antimicrobial activity using the well diffusion method. The crude biosurfactant was tested against Proteus mirabilis, *Micrococcus luteus*, *Klebsiella pnemoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis*. Each isolate was inoculated on sterile Muller-Hinton agar plates. A sterile cork-bore of diameter 7.0 mm was used to make wells on the agar plates. Each well was filled with 50 µL of the supernatant. Distilled water was added to another plate as control. The plates were incubated at 30°C for 24 h. The presence of a clear zone indicated antibacterial activity of the crude biosurfactant [21].

7) Degradation of hydrocarbon by biosurfactant producer

The potential of biosurfactant producers for hydrocarbon degradation was investigated in liquid culture conditions. Isolates were cultivated in mineral liquid medium containing (g L^{-1}): 0.7 KCl, 2.0 KH_2PO_4, 3.0 Na_2HPO_4, 1.0 NH_4NO_3 and trace element solution (4.0 $MgSO_4$, 0.2 $FeSO_4$, 0.2 $MnCl_2$ and 0.2 $CaCl_2$). Mineral salt medium was supplemented with diesel oil (5% v/v) as a source of carbon and energy, an inoculum load of 0.9 optical density, incubated at 30°C for 10 d at 160 rpm. Degradability was assessed by monitoring optical density and pH [22].

8) Statistical analysis

Data obtained were subjected to analysis of variance and means were separated by Duncan's Multiple Range Test using SPSS v. 20.0 ($P<0.05$).

Results and discussion

1) Isolation and characterization of rhizospheric bacteria

Soil serves as a reservoir for microorganisms, but soil incorporation of biochar further boosts microorganism populations. The abundance of microorganisms in biochar-enhanced soil is

consistent with the report of Elad et al. [23] who reported that biochar is a fertilizer, an enricher and a soil conditioner. It is likely that the organisms were able to utilize and degrade both organic and inorganic molecules in the biochar-enhanced soil. Nine rhizospheric bacteria were recovered from the soil without biochar (Bacillus spp; 33.3%, *Pseudomonas* spp; 22.2%, *Citrobacter* spp; 11.1%, *Enterobacter* spp; 22.2% and *Corynebacterium* sp;11.1%). Figure 1(a) and Figure 1(b) show the occurrence of 14 rhizospheric bacteria isolated from sawdust biochar soil (*Bacillus* spp; 28.57, *Staphylococcus* sp; 7.14, *Alcaligenes* spp; 14.29, *Flavobacteria* sp; 7.14, *Azotobacter* sp; 14.29, *Citrobacter* sp; 7.14, Micrococcus sp;7.14, *Klebsiella* sp; 7.14, *Proteus* sp; 7.14) %.

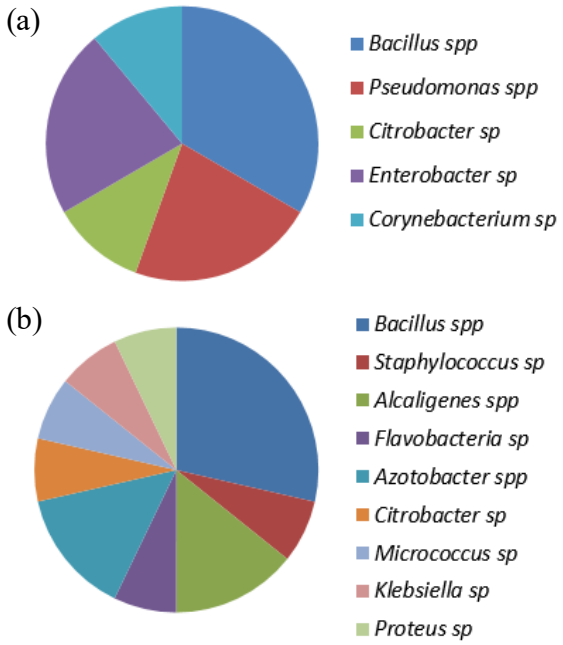

Figure 1 Percentage occurrence of the rhizospheric bacteria in the (a) soil and (b) biochar soil

2) Screening and selection of rhizospheric isolates for biosurfactant production

Of the twenty-three rhizospheric bacteria isolated, two isolates exhibited potential biosurfactant producers following a screening test using complementary (hemolytic, drop collapse, oil spreading, methylene blue method, adhesion method) and confirmatory (emulsification) screening methods. Table 1 shows the complementary screening results and Table 2 shows the confirmatory screening results of the different methods carried out on the rhizospheric bacteria. Interestingly, two isolates that tested positive for the drop collapse method also emerged as potential biosurfactant producers in the emulsification test. The drop collapse test is not as sensitive as the oil displacement test in detecting low levels of biosurfactant production. Youssef et al. [24] recommended the drop collapse assay as a reliable technique for screening biosurfactant production. The drop collapse results is consistent with the work of Said et al. [25] who reported negative results for some isolates and suggested that microorganisms recording negative in the drop collapse test are not good emulsifiers. The methylene blue method is a quantitative assay for detection of anionic surfactants and extracellular glycolipids. The microbes that secrete anionic surfactants were surrounded by dark blue halos [26]. *Alcaligenes faecalis* was the only isolate showing potential to produce an anionic biosurfactant. According to Ariech and Guechi [11], a good biosurfactant producer must have higher than 50% emulsification ability. Following the report by Zou et al. [27] that all biosurfactants are good emulsifiers, two isolates, *Alcaligenes faecalis* and *Bacillus subtilis* were selected as potential biosurfactant producers due to their excellent performance in all the screening methods. However, *Alcaligenes faecalis* was selected over *Bacillus subtilis* due to its higher emulsification index of 65.9%. It was also observed that some of the isolates revealed good emulsification ability with negative results in one or more of the complementary techniques; however, this result was also reported by Sharma et al. [28] and Santhini and Parthasarathi [29].

Table 1 Screening rhizospheric microorganisms for biosurfactant production

Treatments	Isolates	Haemolytic zone (mm)	Drop collapse	Blue	Oil spreading (mm)	Adhesion to hydrocarbon (%)
Sawdust biochar amended soil	*B. subtilis* 1	11.8± 0.40	-	-	10.5 ± 0.8	-
	Alcaligene spp	-	-	-	-	-
	Klebsiella oxytoca	-	-	-	6.2±1.8	20.5±3.0
	Azotobacter chroococcum	-	-	-	-	-
	B. subtilis 2	-	-	-	-	-
	Citrobacter freundii	15.3±0.6	-	-	1.8±0.3	-
	Flavobacterium sp	-	-	-	-	
	Alcaligenes faecalis	23.0 ±0.6	+	0.9± 0.1	15.6±0.3	53.8 ±0.0
	B. sphaericus	6.5±0.0	-	-	1.82±0.35	-
	Azotobacter chroococcum	-	-	-	-	-
	Micrococcus luteus	-	-	-	1.15±0.1	-
	S. saprophyticus	-	-	-	-	
	B. subtilis 3	11.7±0.6	-	-	12.7±0.4	27.0 ±0.1
	Proteus mirabilis	-	-	-	-	-
Unamended Soil	*Enterobacter cloaca*	-	-	-	-	-
	Klebsiella sp		-	-	2.15±0.15	-
	B. subtilis	11.8±0.3	+	-	13.7±1.6	51.5± 2.5
	B. subtilis	-	-	-	-	-
	Klebsiella sp	-	-	-	-	-
	Citrobacter sp.	1.7±0.0	-	-	9.1±0.4	15.0± 4.0
	Corynebacterium sp	-	-	-	-	-
	Bacillus subtilis	1.4±0.1	-	-	-	
	E. cloaca	1.1±0.2	-	-	-	-

Values are Mean ± Standard error of means

Table 2 Emulsification index of bacterial isolates

Isolates	Emulsification index (%)							
	3 h	6 h	9 h	12 h	15 h	18 h	21 h	24 h
Alcaligenes faecalis (SW)	64.6±3.2	66.1±4.1	63.5±2.6	64.5±6.4	65.9±2.1	65.8±4.8	65.9±3.2	65.9±2.9
B. subtilis (SW) 1	68.5±1.4	62.4±0.8	60.3±4.9	54.2±1.3	53.2±7.5	54.6±9.6	54.7±4.9	54.7±5.3
B. subtilis (SW) 2	60.6±7.5	63.6±1.8	61.5±4.6	60.2±6.8	62.4±3.1	61.5±2.8	62.5±2.0	60.0±4.3
B. sphaericus (SW)	52.3±2.1	54.5±4.2	50±3.1	48.6±9.7	46.5±7.3	45.0±2.4	45.0±3.4	44.0±2.5
B. subtilis (S)	56.3±2.4	55.9±1.3	56.0±4.8	56.7±1.4	57.7±2.8	57.1±4.9	57.1±5.3	57.14±2.9
Citrobacter sp. (S)	41.0±1.2	40.5±1.9	40.7± 2.1	42.4±3.0	42.3±1.8	41.6±0.7	43.0±1.4	40.7±2.6

Key: S= Isolate from soil sample; SW= Isolate from sawdust biochar soil sample

Alcaligenes faecalis was identified as a potential isolate because it performed excellently in the screening methods and was therefore selected for biosurfactant production.

3) Biosurfactant production by *Alcaligenes faecalis*

Biosurfactant produced by *Alcaligenes faecalis* using different recovery techniques identified chloroform and methanol as the best extraction solvents with the highest yield of 3.62±0.09, while ethyl acetate produced the lowest (1.37±0.16) yield of biosurfactant (Table 3). Polar and non-polar solvents were used in the study. Their different polarities influence final yield of biosurfactant. According to Qomarudin and Edwan [30], chloroform mixed with methanol in various ratios allows adjustment of the polarity of the extraction agent. The quantity of biosurfactant produced in this study did not corroborate with the findings of Varadavenkatesan and Ramachandra [31] whose highest quantity of biosurfactant produced after 4 d of fermentation was 0.64 g L^{-1}. The disparity in the quantity of biosurfactant produced could be due to the different isolation sites, ratio of chloroform: ethanol and microorganism used.

4) Antimicrobial activity of the biosurfactant

Singh and Cameotra [32] reported that some biosurfactants are suitable alternatives to synthetic medicines and antimicrobial agents and may be useful as safe and effective therapeutic agents. Table 4 shows the antimicrobial activity of the crude biosurfactant produced by *Alcaligenes faecalis* with the highest zone of inhibition by *Micrococcus luteus* and lowest zone of inhibition by *Escherichia coli* (*E. coli*). Both Gram-negative and Gram-positive bacteria were sensitive to the biosurfactant, indicating the potential utility of this biosurfactant in clinical applications.

Table 3 Biosurfactant production using different extraction solvents

Solvent	Dried biosurfactant (g L^{-1})
Acid precipitation	1.84±0.04
Acetone	2.18±0.11
Dichloromethane	1.74±0.07
Chloroform /methanol (2:1)	3.62±0.09
Ethyl acetate	1.37±0.16

Values are Mean ± Standard error of means

Table 4 Antimicrobial activity of crude biosurfactant produced by *Alcaligenes faecalis*

Test Organisms	Zone of inhibition (mm)
Micrococcus luteus	12.4±0.35
Proteus mirabilis	10.0±1.13
S. aureus	8.3±0.4
K. pneumonia	9.8±0.29
E. coli	7.2±1.67
Enterococcus faecalis	9.1± 2.35

Values are Mean ± Standard error of means

5) Degradation of hydrocarbon

In the degradation experiment, absorbance increased and pH decreased over the 10 d study period, suggesting the microorganism's ability to utilize and degrade the diesel oil. Figure 2 shows the degradation analysis of diesel oil. The increase in optical density implies that the bacterial enzymes were able to metabolize the hydrocarbon, releasing acidic metabolites into the medium, thus also lowering the pH over time. Other simple methods for monitoring degradation include the plate count method and measurement of physical and chemical parameters. Previous work on hydrocarbon-degrading bacterial communities have been conducted using GC-MS or HPLC analysis; however, it has been observed that most of the isolates exhibiting better result in GC-MS or HPLC analysis had higher turbidity or optical

density. The method of observing the absorbance or optical density to determine degradation efficiency corroborates the work of Farag and Soliman [33], Xue et al. [34] and Chen et al. [10] where petroleum degradation efficiency was determined by spectrophotometric method and GC-MS analysis.

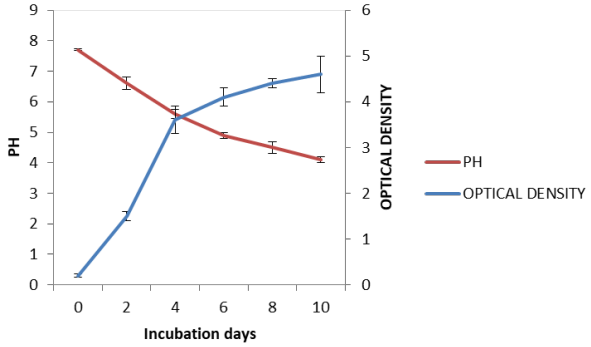

Figure 2 Degradation of hydrocarbon by biosurfactant producer.

6) Phylogenetic analysis of *Alcaligenes faecalis*

Alcaligenes faecalis had previously been considered as a harmless saprophyte in the human intestinal tract but is also common in water, soil and environment [35]. Some *Alcaligenes faecalis* produce enzymes that degrade organic contaminants; these find wide application in the pharmaceutical industry as well as in biodegradation of organic pollutants, sewage and industrial wastewater treatment [36-37]. The phylogenetic tree constructed for the 16s rRNA sequences of A. faecalis from rice-husk biochar soil of okra plant with seven *A. faecalis* GenBank isolates showed that the *A. faecalis* from this study was genetically closely related to *A. faecalis* strains isolated from activated sludge (KY500593.1) in India, sugarcane molasses based distillery waste disposal site (FJ581029.1) in Italy and abattoir waste water (KY345400.1) in Nigeria (Figure 3). This implies that the *A. faecalis* from rice-husk biochar soils of okra plant in Nigeria may be widely distributed and can be isolated from wastewater and soil.

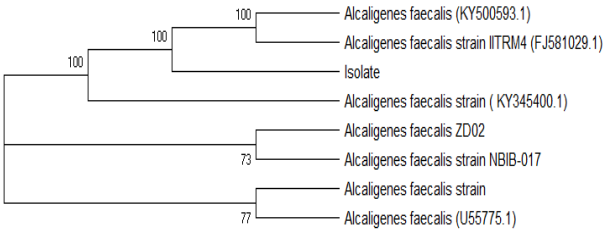

Figure 3 Maximum likelihood tree constructed for the *A. faecalis* from biochar soil with reference to *A. faecalis* strains obtained from the GenBank database using the Tamura-Nei substitution model with bootstrap values (>60%) of 1,000 replicates shown at the nodes.

Conclusion

This study compared the biosurfactant potential of rhizospheric bacteria isolated from soil amended with sawdust biochar and soil without amendment. *Alcaligenes faecalis* from sawdust biochar soil was found to be a potent biosurfactant producing bacterium. Also, the study revealed that the quantity of biosurfactant produced is dependent on the extraction solvent used.

Conflict of interest

The authors have no conflict of interest to declare.

References

[1] Banat, I.M., Makkar, R., Cameotra, S.P. Potential commercial applications of microbial surfactants. Applied Microbiology and Biotechnology, 2000, 53, 495-508.

[2] El-Sheshtawy, H.S., Aiad, I., Osman, M. E., Abo-ELnasr, A.A., Kobisy, A.S. Production of biosurfactants by Bacillus licheniformis and Candida albicans for application in microbial enhanced oil recovery. Egyptian Journal of Petroleum, 2016, 25, 293-298.

[3] Mulligan, C.N., Yong, R.N., Gibbs, B.F. Remediation technologies for metal-contaminated soils and groundwater: An

evaluation. Engineering Geology, 2001, 60, 193-207.

[4] Joshi, S., Bharucha, C., Jay, S., Yadav, S., Nerurkar, A., Anjana, J. Biosurfactant production using molasses and whey under thermophilic conditions. Bioresource Technology, 2008, 99(1), 195-199.

[5] Rahman, K.S., Gakpe, E. Production, characterization and applications of biosurfactants-Review. Biotechnology, 2008, 7, 360-370

[6] Ron, E. Z., Rosenberg, E. Natural roles in biosurfactants. Environmental Microbiology, 2011, 3, 229-236.

[7] Sachdev, D.P., Cameotra, S.S. Biosurfactants in agriculture. Applied Microbiology and Biotechnology, 2013, 97(3), 1005-1016.

[8] Jeffery, S., Frank, G.A., Verheijen, M., Bastos, A.C. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis". Agriculture, Ecosystems and Environment, 2011, 144(1), 175-187.

[9] Woldetsadik, D., Drechsel, P., Marschner, B., Itanna, F., Gebrekidan, H. Effect of biochar derived from faecal matter on yield and nutrient content of lettuce (Lactuca sativa) in two constracting soils. Environmental Systems Research, 2017, 6, 2-5

[10] Chen, Q., Li, J., Liu, M., Sun, H., Bao, M. Study on the biodegradation of crude oil by free and immobilized bacterial consortium in marine environment. PLoSONE, 2017, 12(3):e0174445. DOI: 10.1371/journal.pone.0174445

[11] Ariehi, M., Guech, A. Assessment of four different methods for selecting biosurfactant producing extremely halophilic bacteria. African Journal of Biotechnology, 2015, 14 (21), 1764-1772

[12] Loganathan, K., Gaurav, K., Kokati, V., Bhaskara, R. Comparison of methods and screening of biosurfactant producing marine actinobacteria isolated from Nicobar marine sediment. Environmental Biotechnology Division, 2010, 2(1), 34-38

[13] Goulart, G.G., Coutinho, J.O., Monteiro, A.S., Siqueira, E.P., Santos, V.L. Isolation and characterization of gasoline-degrading yeasts from refined oil-contaminated residues. Journal of Bioremediation and Biodegradation, 2014, 5, 1-9.

[14] Satpute, S.K., Bhawsar, B.D., Dhakephalkar, P.K., Chopade, B.A. Assessment of different screening methods for selecting biosurfactant producing marine bacteria. Indian Journal of Marine Sciences, 2008, 37, 243-250.

[15] Kebbouche-Gana, S., Gana, M.L, Khemili, S., Fazouane-naimi, F., Bouanane, N.A., Penninckx, M., Hacene, H. Isolation and characterization of halophilic archaea able to produce biosurfactants. Journal of Industrial Microbiology and Biotechnology, 2009, 36(5), 727-738.

[16] Joshi, M., Deshpande, J.D. Polymerase chain reaction: Methods, principles and applications. International Journal of Biochemistry Research, 2010, 1(15): 81-97.

[17] Pacwa-Płociniczak, M., Anna, G.P., Poliwoda, A., Piotrowska-Seget, Z. Characterization of hydrocarbon-degrading and biosurfactant -producing Pseudomonas sp. P-1 strain as a potential tool for bioremediation of petroleum -contaminated soil. Environmental Science and Pollution Research, 2014, 21, 9385-9395

[18] Saravanan, V., Vijayakumar, S. Isolation and screening of biosurfactant producing microorganisms from oil contaminated soil. Journal of Academia and Industrial Research, 2012, 1(5), 264-268

[19] Qiao, N., Shao, Z. Isolation and characterization of a novel biosurfactant produced by hydrocarbon-degrading bacterium Alcanivorax dieselolei B-5. Journal of Applied Microbiology, 2010, 108, 1207-1216.

[20] Kim, P., Jaewon, R., Young, H., Youn-Tae Chi. Production of biosurfactant lipopeptide iturin A, Fengyan and surfactin A from Bacillus subtilis CMB32 for control of colletotrichum gloeosporoides. Journal of Microbiology and Biotechnology, 2010, 138-145

[21] Mouafia, F.E., Mostafa, M.A., Maysa, E. M. Optimization of biosurfactant production by Bacillus brevis using response surface methodology. Biotechnology Reports, 2016, 9, 31-37.

[22] Marchut Mikolajczyk, O., Drozdzynski, P., Pietrzyk, D., Antczak, T. Biosurfactant production and hydrocarbon degradation activity of endophytic bacteria isolated from Chelidonium majus. Microbial Cell Factories, 2018, 17(171), 1-9.

[23] Elad, Y., Rav, D.D., Meller, H.Y., Borenshtein, M., Kalifa, H.B., Silber, A., Graber, E.R. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. Phytopathology, 2010, 100(9), 913-921.

[24] Youssef, N.H., Duncan, K.E., Nagle, D.P., Savage, K.N, Knapp, R.M., McInerney, M.J. Comparison of methods to detect biosurfactant production by diverse microorganisms. Journal of Microbiological Methods, 2004, 56(3), 339-347

[25] Said, B.H., Latifa, S., Abdeljelil, G., Abderrazak, M. Screening of potential biosurfactant producing bacteria isolated from seawater biofilm. African Journal of Biotechnology, 2012, 11(77), 14153-14158.

[26] Vandana, P., Singh, D. Review on biosurfactant production and its application. International Journal of Current Microbiology and Applied Sciences, 2018, 7(8), 4228-4241

[27] Zou, C., Wang, M. Xing, Y., Lan, G., Ge, T., Yan, X., Gu, T. Characterization and optimization of biosurfactant produced by Acinetobacter baylyi ZJ2 isolated from crude oil-contaminated soil sample toward microbial enhanced oil recovery applications. Biochemical Engineering Journal, 2014, 90, 49-58.

[28] Sharmaa, R., Singhb, J., Verma. N. Production, characterization and environmental applications of biosurfactants from Bacillus amyloliquefaciens and Bacillus subtilis. Biocatalysis and Agricultural Biotechnology, 2018, 16, 132-139

[29] Santhini, K., Parthasarathi, R. Isolation and screening of biosurfactant producing microorganisms from hydrocarbon contaminated soils from automobile workshop. International Journal of Pharmaceutical and Biological Archive, 2014, 5(2), 158-167

[30] Helmy, Q., Kardena, E. Strategies toward commercial scale of biosurfactant production as potential substitute for it's chemically counterparts. International Journal of Biotechnology, 2011, 12, 66-86.

[31] Varadavenkatesan, T., Ramachandra, V. Production of lipopeptide biosurfactant by a novel Bacillus sp. and its applicability to enhanced oil recovery, International Scholarly Research Notices Microbiology, 2013, 1-8.

[32] Singh, P., Cameotra, S. Potential applications of microbial surfactants in biomedical sciences. Trends in Biotechnology, 2004, 22, 142-146.

[33] Farag, S., Soliman, N.A. Biodegradation of crude petroleum oil and environmental pollutants by Candida tropicalis strain. Brazilian Archives of Biology and Technology, 2011, 54(4), 821-830.

[34] Jianliang, X., Yanan, W., Zhixiu, L., Menglu, L., Xiyu, S., Huajun, W., Bing, L. Characteristic assessment of diesel-degrading bacteria immobilized on natural organic carriers in marine environment: the degradation activity and nutrient. Scientific Reports, 2017, 7(8635), 1-9.

[35] Phung, L.T., Trimble, W.L., Meyer, F., Gilbert, J.A, Silver, S. Draft genome sequence of Alcaligenes faecalis sub sp faecalis NCIB8687 (CCUG2071). Journal of Bacteriology, 2012, 194(18), 5153.

[36] Kong, L., Zhu, S., Zhu, L., Xie, H., Wei, K., Yan, T., ..., Sun, F. Colonization of Alcaligenes faecalis strain JBW4 in natural soils and its detoxification of endosulfan. Applied Microbiology and Biotechnology, 2014, 98, 1407-1416.

[37] Xia, Z. Effect of tween 80 on the production of curdlan by Alcaligenes faecalis ATCC 31749 Carbohydrate Polymers, 2013, 98, 178-180.