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RESEARCH ARTICLE

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Production of Rhamnolipids by *Pseudomonas aeruginosa* HB6 (39) Isolated from Petroleum-Hydrocarbon Contaminated Environment

Faith C. Samuel-Osamoka, Daniel J. Arotupin, Oladipo O. Olaniyi, and Ibrahim M. Banat

ABSTRACT

The current study evaluated hydrocarbon-degrading and biosurfactant-producing potentials of bacteria isolated from hydrocarbon contaminated soil in Nigeria, the largest crude oil reservoir in Africa. Pure bacterial isolates were grown on nutrient and Bushnell Haas agar. Bacterial isolates that grew on both media were molecularly identified via 16S rDNA sequence. Biosurfactant production detection was carried out via oil spread test, drop collapse test and surface tension measurement. The bacterial isolate with the lowest surface tension value was used for further studies. The growth of the selected isolate was measured using Spectroscopic technique, while the production of biosurfactants in the culture supernatant were determined by the measurement of surface tension, and the extracted surfactants were characterized by High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS). The colony forming unit on nutrient agar ranged from 1.35 x 10^8 to 1.93 x 10^8 cfu/g while the colony forming unit on Bushnell Haas agar ranged from 9.33 x 10^5 to 1.84 x 10^6 cfu/g. The bacterial species belonged to three genera including *Bacillus*, *Cellulosimicrobium* and *Pseudomonas*. *P. aeruginosa* HB6 (39) with accession number MW367569.1 had the lowest surface tension value (33.77±0.12a) indicating that it was the best biosurfactant producer. The test isolate attained early stationary phase at 10h and the cell-free supernatant showed excellent surface tension reduction potential. The extracted biosurfactant contained ample mono and di-rhamnolipid congeners. *P. aeruginosa* HB6 (39) is a potential bioremediation agent and can also be used for large scale production of rhamnolipids for other industrial applications.

Keywords: Bacteria, biodegradation, biosurfactants, petroleum-hydrocarbon, rhamnolipids, surface tension.

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I. Introduction

The activities related to the exploration and exploitation of crude oil are responsible for environmental pollution most especially in oil producing countries and these pollutions causes great risk to abiotic and biotic component of the ecosystems (Odokuma & Dickson, 2009; Das and Chandran, 2011; Irving *et al*., 2012). In Nigeria, energy used for industrial and domestic purposes is derived from unrefined petroleum-based products which are equally the backbone of economic development (Urhibo & Ejechi, 2017). However, unregulated leakages and accidental spillage of these products into aquatic and terrestrial environment polluted, paralyzed agricultural activities, and rendered many jobless (Moussa *et al*., 2014; Urhibo & Ejechi, 2017). In addition, the effect of petroleum pollutants in plant and animal tissues might cause death or extensive local system damage resulting from deleterious mutations (Das & Chandran, 2011). The impact of these pollutants on the environment is the driving force behind the creation of many decontamination processes. Remediation of hydrocarbon contaminated soils is mainly addressed via chemical and biological agents or their metabolites. Beside the cost implication that characterized the use of chemicals such as synthetic surfactants in the degradation of these pollutants, they may be toxic to all components of the ecosystem or probably result in the generation of secondary pollutants of more detrimental effect (Abdel-Mawgoud *et al*., 2010; Shreve & Makula, 2019). For these reasons, biological methods involving the use biosurfactants from microbial sources which is less capital intensive and more ecologically friendly seems to be a promising approach for the restoration of sites contaminated with petroleum products (Moussa *et al*., 2014). The advantages of biosurfactants over conventional chemical surfactants include better environmental compatibility, lower toxicity, appreciable degradability, good foaming potentials, high specificity, and better performance at extreme environmental conditions (Abdel-Mawgoud *et al.,* 2010).

Arrays of microorganisms from hydrocarbon contaminated soils have been extensively evaluated for biosurfactants production with no attention paid to organisms 'from contaminated soils from Nigeria, the largest oil producing country in Africa where oil spillage is not moderated by government policy. In previous studies, evaluation of bacteria for biosurfactants production was limited to hydrocarbon contaminated soil (Bento *et al.,* 2005; Beulah, 2018) whereas microbial contents of uncontaminated soil remain unexplored.

Biosurfactants are useful compounds produced by microorganisms which could be cell-bound or extracellular (Mulligan, 2005). The presence of hydrophobic and hydrophilic parts in these biomolecules enhances their accumulation at interface amid two immiscible liquids which leads to a reduction in surface and interfacial tensions as well as formation of micro-emulsion (Banat *et al*., 2010). They are suitable alternatives to the chemically synthesized surfactants which are currently the most commonly used surface-active agents (Marchant & Banat, 2012). The multidimensional properties of biosurfactants include surface tension reduction, greater solubility, detergency potential, wetting and foaming abilities (Fadhile *et al.,* 2016; Banat *et al*., 2021). These accounts for their application in various industries (food, cosmetics, pharmaceutical and petroleum) and also in the biological treatment of polluted areas (Bhattacharya *et al*., 2017; Naughton *et al.,* 2019).

Microbial surfactants are categorized based on the type of microorganisms producing them as well as their chemical nature and composition (Banat *et al*., 2010; Vijayakumar & Saravanan, 2015), and as such have been broadly divided into two groups; the biosurfactants with low molecular mass and the bioemulsifiers with higher molecular mass (Karanth *et al.,* 1999; Mulligan, 2005; Banat *et al.,* 2021). Differing phenotypic properties can be attributed to these groupings, for example, biosurfactants possess tendency of lowering surface tensions but bio-emulsifiers serves as emulsion stabilizing agents (Karanth *et al.,* 1999; Banat *et al.,* 2010). The glycolipids are a well-studied class of biosurfactants and include the rhamnolipids, trehalose lipids, and sophorolipids (Bodour *et al.,* 2003; Perfumo *et al*., 2013). Rhamnolipids are made up of either a single or double molecules of rhamnose connected to either a single or double molecules of ß-hydroxydecanoic acid. (Desai and Banat, 1997). Rhamnolipids produced by bacteria is widely reported in the literature (Mulligan, 2005; Toribio *et al*., 2010; Chebbi *et al*., 2017; Shreve & Makula, 2019).

The most implicated bacteria in the production of rhamnolipids are the *Pseudomonas* species (Abouseoud *et al*., 2008; Marchant and Banat, 2012; Sinumvay and Ishimwe, 2015; Baskaran *et al.,* 2020). *P. aeruginosa* species produces rhamnolipids composed of one or two rhamnose molecules linked to a single or double 3-hydroxy fatty acids molecules and are therefore classified as monorhamnolipids or dirhamnolipids (Siddhartha *et al*., 2006; Irfan-Maqsood and Seddiq-Shamma, 2014). The properties of rhamnolipids include biodegradability (Lima *et al.,* 2011; Rodriguez-Lopez *et al.,* 2019), lowering of surface and interfacial tension (Marchant and Banat, 2012), and ability to emulsify various hydrocarbons (Abalos *et al*., 2001). They are also non-hazardous and do not pose any mutagenic threat to living organisms (Voulgaridou *et al.,* 2021).

Recent increase in the application of rhamnolipids for industrial and bioremediation purposes is attributed to their diverse properties. Sekhon & Rahman (2014) reported that several companies in the USA now produce rhamnolipids at commercial scale and these products are used as pharmaceutical, cosmetic, antimicrobial, anticancer, and cleaning products. Jeneil Biosurfactant Co. (USA) produces biosurfactants applicable in agriculture; bioremediation and oil recovery processes (Banat *et al*., 2010). Despite the growing interest and successes recorded in the production of biosurfactants, the current challenge facing large scale production for marketable purposes is the highly expensive production cost and low yield when compared with the chemically synthesized surfactants. Therefore, there is a need to explore more strains of microorganisms, most especially indigenous organisms from Nigeria soil samples, one of the largest crude oil producing country in the world with better yields. This study aimed at identifying hydrocarbon-degrading bacteria obtained from petroleum-hydrocarbon contaminated soils, screening for rhamnolipids production and characterizing the rhamnolipids produced. This is a way of increasing the possible sources of biosurfactant producing microorganisms which would ultimately results in complete replacement of synthetic surfactants for bioremediation purposes.

II. Materials and Methods

A. Sample Collection

Soil samples contaminated with petroleum hydrocarbon were collected at a depth of 10-15 cm using soil auger from 5 automobile workshops in Shasha, Akure, Ondo State, Nigeria. The collected samples were transferred to pre-sterilized bottles and immediately taken to the laboratory where they were preserved at 4°C in lieu of more study.

B. Isolation and Enumeration of Total Heterotrophic Bacteria (THB) and Total Hydrocarbon Degrading Bacteria (HDB)

Total heterotrophic bacterial population was enumerated by pour plate method as described by Chikere *et al*. (2009) while total Hydrocarbon degrading bacteria population were enumerated by spread plate technique using Bushnell Haas medium comprising 1% crude oil. The Petri-dish inoculated with the samples were incubated at 25°C for one week. Repeated sub-culturing gave rise to pure cultures. Pure isolates were inoculated by streak method on Bushnell Haas agar Petri-dishes overlaid with 100 microliters of crude oil as well as on freshly prepared nutrient agar Petri-dishes. These were then placed in the incubator at 25°C for two weeks and 24-48 h respectively. Isolates that grew on NA plates only were regarded as non-degraders of crude oil, while isolates that grew on both media were confirmed as hydrocarbon degraders and stored in NA slants at 4ºC for further studies (Udotong *et al*., 2008).

C. Characterization and Identification of Bacterial Isolates

The DNA of pure isolates was extracted using DNeasy

ultraclean bacterial kit (Qiagen) following the protocols stated by the manufacturer. The primers previously used by Muhling *et al*. (2008) were used to amplify the extracted DNA based on polymerase chain reaction (PCR), these were 96fm (5^1- GAGTTTGATYHTGGCTAG-3^1) and 1152 UR (5^1 ACGGHTACCTTGTTACGACTT-3^1). The separation of the PCR products based on their molecular weight was caried out using 1% (w/v) agarose gel prepared with TBE buffer as described by Twigg *et al*. (2018). SybrSafe DNA stain (Thermo Fisher Scientific) was used to visualise the DNA under UV light. Wizard Gel and PCR Clean-up System (Promega) was used to purify the amplified DNA which were then sequenced using Sanger sequencing technique by Eurofins Genomic services, Germany. All sequence data were analysed by blastn online comparison for identification of the strains. Accession numbers were obtained after the DNA sequence were submitted to the GeneBank Centre (NCBI, USA).

D. Screening of Isolates for the Production of Biosurfactant.

1) Oil-spreading test

To apply the oil-spreading test, 10 µl of crude oil was added to the surface of 40 ml of distilled water into a Petri dish to form a thin oil layer. A further 10 µl of the culture supernatant was gently dropped on the center of the oil layer. After one minute, if biosurfactant was present in the supernatant, the oil is displaced, and a clearing zone was formed as described by Morikawa *et al*. (2000). The diameter of the clear zone on the oil surface was measured in 2 replications for each isolate. A water drop was used as a negative control.

2) Drop collapse test

The drop collapse assay relies on the destabilization of a liquid drop on the hydrocarbon surface by cell-free extract containing biosurfactant. The test was carried out as described by Jain *et al.* (1991). A drop of the culture supernatant was placed carefully on an oil coated glass slide and observed after one minute, biosurfactant production was considered positive if the drop of supernatant collapses and spread on the oil coated surface. Meanwhile, if the drop remains after one minute it was considered negative. This test was simultaneously carried out using distilled water as a control.

3) Surface tension measurement

The assay for the ability of strains used in this study to reduce surface tension was determined based on De Noüy ring method via digital tensiometer (K10T, Kruss, Germany). A glass beaker containing five milliliters (5 ml) of the culture supernatant was positioned in the tensiometer stage, and the surface tension of the sample measured by submerging a platinum wire ring into the sample and allowing it to pull through the air-liquid interface (Bodour & Miller-Maier, 1998). Readings were taken in triplicate and the average recorded.

E. Determination of the Bacterial Growth and Surface Tension Reduction

The test organism was cultured in a modified mineral salt media. The optical density and surface tension of the culture medium were determined. Each isolate was activated by introducing a single colony into a tube containing 10 ml of nutrient broth which was then placed in an incubator at 30°C for 18-24 h, this served as a seed culture. 200 ml mineral salt medium was inoculated with the seed culture at a 2% final volume and incubated at 30°C for 96 h at 200 rpm, 7 ml of culture media was taken at 6 h intervals for up to 96 h. The optical density of 2 ml of production media was determined at 600 nm via spectrophotometer while the remaining 5 ml was centrifuged, and the supernatant used for surface tension assay.

F. Production, Extraction and Characterization of Biosurfactants

The production culture medium was poured into a tube and centrifuged at 10,000 rpm for 20 minutes at 4°C after which Whatman qualitative filter paper grade 1 was used to filter the upper layer which is the cell-free supernatant. The pH of the filtered supernatant was adjusted to 2.0 by adding drops of 1 M HCl and kept over night at 4°C to allow acid precipitation of the surfactants. The surfactant was then extracted thrice via liquid phase extraction using ethyl acetate. A mixture of same volume of the supernatant and ethyl acetate was added into a separating funnel, shaken vigorously, and allowed to separate into two distinct layers, the layer containing the surfactant was dried under vacuum in a rotary evaporator at 45°C (Irorere *et al.,* 2017). The product was re-constituted in methanol at the concentration of 1mg/ml and characterized using High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) (Smyth *et al.,* 2010). The various components in the extracted biosurfactants were identified and the spectra compared with the NIST Library compounds.

G. Data Analysis

The data obtained for the enumeration of total heterotrophic and hydrocarbon degrading bacteria as well as oil spread, and emulsification index (E_{24}) tests were analyzed using statistical package for social sciences (SPSS). The experiment was done in triplicate and all measurements were expressed as mean ± standard deviation. The criterion for significance was set at $p \leq 0.05$. All measurements obtained for optical densities and surface tension were analyzed using Graph pad prison version 5. The experiments were done in duplicate.

III. Results

A. Total Heterotrophic Bacteria Count on Nutrient Agar and Total Hydrocarbon-degrading Bacteria on Bushnell Haas Agar

The total heterotrophic bacteria count ranged from 1.35 x 10^8 to 1.93 x 10^8 cfu/g with soil sample B having the highest total heterotrophic bacterial count of 1.93 x 10^8 cfu/g while sample D had the lowest count of 1.35 x 10^8 cfu/g as presented in Table I. The result also showed the total hydrocarbon-degrading bacterial load on Bushnell Haas medium ranged from 9.33 x 10^5 to 1.84 x 10^6 cfu/g. Similarly, sample B had the highest hydrocarbon-degrading bacterial

population of 1.84 x 10^6 while sample D had the lowest hydrocarbon degrading bacterial population of 9.33 x 10^5 cfu/g. There was no significant difference between the total heterotrophic bacteria population in sample A and C, however, the hydrocarbon-degrading bacteria population in sample A (1.48 x 10^6) was higher than sample C (1.37 x 10^6). The result in Table I revealed that the bacterial population was higher in sample B while sample D had the lowest bacteria population.

TABLE I: TOTAL HETEROTROPHIC BACTERIA COUNT ON NUTRIENT AGAR AND TOTAL HYDROCARBON-DEGRADING BACTERIA ON BUSHNELL HAAS AGAR

Sample Code	Nutrient agar (cfu/g)	Bushnell Haas agar (cfu/g)
A	1.62 x 10^8 ±1.45^c	1.48x 10^6 ±0.33^c
B	1.93 x 10^8 ±1.15^d	1.84 x 10^6 ±0.88^e
C	1.60 x 10^8 ± 0.58^c	1.37 x 10^6 ± 0.88^d
D	1.35 x 10^8 ±0.57^a	9.33 x 10^5±0.88^a
E	1.42 x 10^8 ±1.15^b	1.22 x 10^6±0.58^b

Values along the column are the mean of 3 determinations and those with the same superscript are not significantly different, $p \leq 0.05$. A, B, C, D, E- represents samples from 5 contaminated sites.

B. Molecular Identities of Bacterial Isolates Obtained from Soil Samples Contaminated with Petroleum-Hydrocarbon

The result presented in Table II, showed the molecular identities of eighteen potential hydrocarbon-degrading bacteria isolated from petroleum-contaminated soil samples. These includes; *Bacillus safensis* HA8, *Cellulosimicrobium cellulans*, *Bacillus safensis* HA10, *Bacillus safensis* HA15, *Bacillus safensis* HB1, *Bacillus safensis* HB1(N), *Pseudomonas aeruginosa* HB6(39), *Cellulosimicrobium cellulans* HC9, *Pseudomonas stutzeri* HC9(N), *Cellulosimicrobium cellulans* HC10, *Bacillus safensis* HC21 *Bacillus cereus* HD3, *Bacillus pumilus* HD4, *Bacillus cereus* HD7, *Bacillus safensis* HE4, *Bacillus pumilus* HE4(N), *Bacillus cereus* HE5, *Bacillus pumilus* HE7. The result revealed that the percentage similarities of all the isolates were above 99%. The accession numbers of all the isolates obtained from the gene bank were also presented.

C. Screening for Biosurfactant Producers

The result of the screening for biosurfactants producers is presented in Table III. The cell free supernatant of all the bacterial isolate showed positive results for oil spread test with the zone of clearance ranging from 10.00 ± 1.00^b to 14.67 ± 1.20^c mm in diameter. *Bacillus safensis* HB1 had the highest zone of clearance of 14.67 ± 1.20^c mm followed by *Pseudomonas aeruginosa* HB6 (39) with the zone of clearance 12.00 ± 1.15^b mm. *Bacillus pumilus* had the lowest zone of clearance of 10.00 ± 1.00^b mm. The control medium showed no zone of clearance indicating the absence of biosurfactant production. The drop collapse test showed that four out of the 5 bacterial isolates were positive while one bacterial isolate identified as *Bacillus pumilus* showed negative result. The drop collapse test for *B. safensis* HB1 and *P. aeruginosa* HB6 (39) showed very strong positive results. Furthermore, the measurement of surface tension of the culture medium inoculated with *P. aeruginosa* HB6 (39) had the lowest surface tension measurement value of 33.77 ± 0.12^a mN/m followed by the surface tension of the medium inoculated with *B. safensis* HB1 (48.83 ± 1.78^b mN/m). *P. aeruginosa* HB6 (39) reduced the surface tension of the medium from 54.57±1.01^{ab} to 33.77±0.12^a while *B. safensis* reduced the surface tension of the medium from 58.43±1.66^b to 48.83±1.78^b. The medium inoculated with *Bacillus pumilus* showed no reduction in surface tension measurement indicating the absence of biosurfactant production.

TABLE II: MOLECULAR IDENTITIES OF ISOLATES OBTAINED FROM PETROLEUM-HYDROCARBON CONTAMINATED SOIL SAMPLES

S/N	Isolate codes	Bacterial isolates	Percentage similarities (%)	Accession number
1.	HA8	*Bacillus safensis* HA8	99.9	MW367558.1
2.	HA9	*Cellulosimicrobium cellulans* HA9	99.4	MW367559.1
3.	HA10	*Bacillus safensis* HA10	99.6	MW367560.1
4.	HA15	Bacillus safensis HA15	99.7	MW367561.1
5.	HB1	*Bacillus safensis* HB1	99.7	MW367568.1
6.	HB1N	*Bacillus safensis* HB1(N)	99.6	MW367567.1
7.	HB6(39)	*Pseudomonas aeruginosa* HB6(39)	99.1	MW367569.1
8.	HC9	*Cellulosimicrobium cellulans* HC9	99.3	MW367574.1
9.	HC9(N)	*Pseudomonas stutzeri* HC9(N)	99.4	MW367573.1
10.	HC10	*Cellulosimicrobium cellulans* HC10	99.6	MW367575.1
11.	HC21	*Bacillus safensis* HC21	99.5	MW367576.1
12.	HD3	*Bacillus cereus* HD3	100	MW367583.1
13.	HD4	*Bacillus pumilus* HD4	99.3	MW367584.1
14.	HD7	*Bacillus cereus* HD7	99.6	MW367585.1
15.	HE4	*Bacillus safensis* HE4	99.9	MW367591.1
16.	HE4(N)	*Bacillus pumilus* HE4(N)	99.8	MW367590.1
17.	HE5	*Bacillus cereus* HE5	99.9	MW367592.1
18.	HE7	*Bacillus pumilus* HE7	99.4	MW367593.1

TABLE III: BIOSURFACTANT PRODUCTION BY SELECTED BACTERIAL ISOLATES

Bacterial isolate	Oil Spread Test (mm)	Drop collapse	Surface tension at 0 h (mN/m)	Surface tension at 48 h (mN/m)
Control	0.00±0.00a	-	65.13±1.60^c	65.80±0.30^c
Bacillus safensis HB1	14.67±1.20^c	+++	58.43±1.66^b	48.83±1.78^b
Pseudomonas aeruginosa HB6 39	12.00±1.15^b	+++	54.57±1.01^{ab}	33.77±0.12^a
Cellulosimicrobium cellulans HC9	10.33±1.00^b	+	55.60±1.19^{ab}	49.97±0.54^{bc}

Bacillus cereus HE5	11.33±0.88^{b}	+	56.07±1.76^{ab}	51.53±1.07cd
Bacillus pumilus HD4	10.00±0.33^{b}	-	51.57±0.76a	52.77±0.19^{d}

TABLE IV: Chemical Characteristics of Biosurfactants Produced by Pseudomonas aeruginosa HB6 (39)

Retention time (min)	Pseudomolecular ion [M-H]$^{-}$	Molecular Weight	Molecular formula	Rhamnolipids congener	Abundance (%)
5.26	464.2	465.2			0.97
11.21	222.8	223.8			2.34
11.24	196.7	197.7			1.16
13.36	407.2	408.2			0.46
13.49	593.0	594.0	$C_{28}H_{50}O_{13}$	Rha-Rha-C_8-C_8	0.03
14.69	448.3	449.3			0.94
15.59	621.1	622.1	$C_{30}H_{54}O_{13}$	Rha-Rha-C_8-C_{10}	6.29
17.51	649.1	650.1	$C_{32}H_{58}O_{13}$	Rha-Rha-C_{10}-C_{10}	25.49
19.02	675.1	676.1	$C_{34}H_{60}O_{13}$	Rha-Rha-$C_{12:1}$-C_{10}	4.34
20.36	677.1	678.1	$C_{34}H_{62}O_{13}$	Rha-Rha-C_{12}-C_{10}	8.38
20.48	503.1	504.1	$C_{26}H_{48}O_9$	Rha-C_{10}-C_{10}	36.65
22.06	529.1	530.1	$C_{28}H_{50}O_9$	Rha-C_{10}-$C_{12:1}$	6.11
23.19	531.0	532.0	$C_{28}H_{52}O_9$	Rha-C_{10}-C_{12}	5.87

Values along the column are the mean of 3 determinations and those with the same superscript are not significantly different, $p \leq 0.05$. + = positive, - = negative, control = (Un-inoculated medium)

D. Growth and Surface Tension Reduction Pattern of P. aeruginosa HB6 (39)

The growth pattern and the ability to reduce surface tension by *P. aeruginosa* HB6 (39) in growth media are shown in Fig. 1. The growth curves showed that the isolate attained the stationary phase quite early at 10 h, this was sustained throughout the growth period. Similarly, the significant reduction in the surface tension value occurred at 10 h and was sustained throughout the incubation period. The optical density for the bacterial culture of *P. aeruginosa* HB6-39 ranged from 0.09-1.34 nm with the highest optical density value recorded at 24 h, the surface tension measurement value of the fermentation media decreased from 46.4 to 27.7 mN /m with the lowest value obtained at 22 h (Fig. 1).

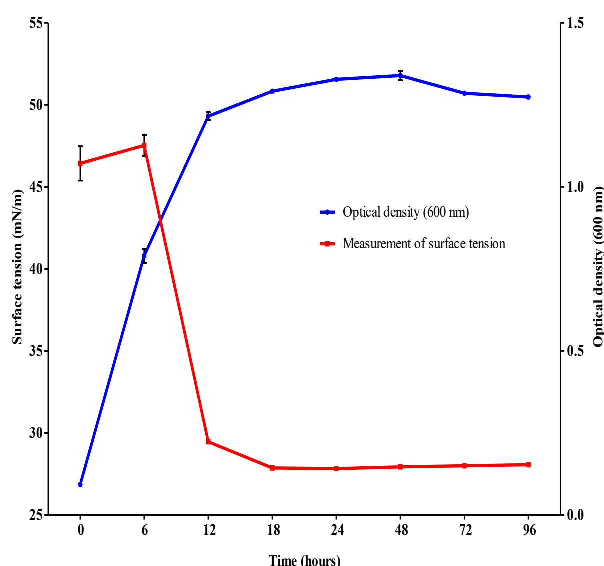

Fig 1. Growth rate and surface tension reduction pattern of *P. aeruginosa* HB6 (39).

E. Chemical Characteristics of Biosurfactants Produced by Pseudomonas aeruginosa HB6 (39)

The chemical characteristics of the extracted biosurfactants are shown in Table II. The pseudomolecular ions of the predominant peaks corresponded to the molecular weight as well as molecular formular of different rhamnolipids congeners as listed in Table II. The length of the fatty acid chain in all the rhamnolipids congeners in this analysis ranged from 8 to 12 carbon The mono-rhamnolipids congener present in the extracted surfactants were; Rha-C_{10}-C_{10}, Rha-C_{10}-$C_{12:1}$ and Rha-C_{10}-C_{12} while the di-rhamnolipids congeners include; Rha-Rha-C_8-C_8, Rha-Rha-C_8-C_{10}, Rha-Rha-C_{10}-C_{10}, Rha-Rha-$C_{12:1}$-C_{10} and Rha-Rha-C_{12}-C_{10}. It should be noted however, that the molecular weight of some compounds found in the extracted surfactants did not correspond to any rhamnolipids congener. The percentage abundance of mono-rhamnolipids congeners (48.63%) in the surfactant produced by *P. aeruginosa* HB6 (39) was higher than di-rhamnolipids (44.53) with Rha-C_{10}-C_{10} (Mw 504.1) having the highest relative abundance (36.65).

IV. Discussion

The total heterotrophic bacteria count obtained in this study ranged from 1.35 x 10^8 to 1.93 x 10^8 and were higher than that previously documented by Agyeman-Duah *et al*. (2017), who recorded that the average number of total heterotrophic bacteria from petroleum contaminated soils ranged from 1.51 x 10^5 to 1.817 x 10^6 cfu/g. The high number of total heterotrophic bacteria in this study may be due to the organic matter concentration in the soil samples as well as the anthropogenic activities in the sampling areas. The total hydrocarbon-degrading bacteria population obtained in this study ranged from 9.33 x $10^5$to 1.84 x 10^6 cfu/g, this was higher than the total hydrocarbon degrading bacteria population recorded by Subathra *et al*. (2013) which ranged from 5.7 x 10^4 to 1.58 x 10^5 cfu/g. Eighteen bacterial isolates identified via 16S rDNA in this study belonged to three genera including *Bacillus*, *Cellulosimicrobium* and *Pseudomonas*. This confirms previous report stating the occurrence of diverse bacteria species with petroleum-

hydrocarbon degrading ability in oil contaminated soils. For example, Ajayi & Abiola, (2018) identified bacterial species with the ability to degrade crude oil from oil-polluted soils. These bacterial species belonged to the following genera: *Micrococcus*, *Bacillus Pseudomonas* and *Acinetobacter*.

The occurrence and survival of diverse bacteria species in petroleum-hydrocarbon contaminated soils may be due to the ability of microorganisms to breakdown xenobiotic chemicals through selective enrichment and gene modification (Ramdass & Rampersad, 2021). *Bacillus* species were predominant in this study, this agrees with the report of Enaigbe *et al*. (2020), who documented the predominance of *Bacillus* species in oil contaminated soils. *Bacillus* species can survive in harsh environmental conditions due to their ability to form spores which protects them from the toxic compounds found in such environments (Onifade & Abubakar, 2007), this accounts for their high population in soils contaminated with petroleum-hydrocarbon. The occurrence of hydrocarbon degrading *cellulosimicrobium cellulans* in environments polluted with petroleum-hydrocarbons have also been documented (Shaieb *et al*., 2015 and Nkem *et al*., 2019). *Pseudomonas* species are ubiquitous in the environment as previous studies have shown that they occur both in contaminated and undisturbed environments (Bodour *et al*., 2003), this agrees with our observation that *Pseudomonas* species were present in the petroleum-hydrocarbon contaminated soils. *Pseudomonas* species are associated with biosurfactants production (Silva *et al*., 2017), which may be responsible for the ability of these species to survive in oil polluted environment, because biosurfactants serves as emulsifiers enhancing the solubility of the hydrocarbons and resulting in their bioavailability to the microorganisms (Das *et al*., 2014)

The result for the detection of biosurfactant production revealed that all the bacteria tested were positive for oil spread test. This may be due to the sensitive nature of this technique to detect the presence of biosurfactants at low concentration. The sensitivity and suitability of this method over other methods of detecting biosurfactant production have been reported (Hamzah *et al*., 2013; Pradnya & Dhiraj, 2014). The range of the zone of clearance obtained in this study is higher compared to the result obtained by Ndibe *et al*. (2018) where diverse bacteria showed zone of clearance ranging from 5 to 11 mm. This difference may be because the bacterial isolates used for this study were potential hydrocarbon degraders which usually develops additional mechanisms of survival in harsh environmental conditions. This study also revealed that *B. safensis* had the highest zone of clearance of 14.67 mm indicating that this isolate was a potential surfactant producer. This is in agreement with the report of Hanano *et al*. (2017), who documented that *Bacillus safensis* showed an increase in the hydrocarbon clearing zone ranging from 10 to 22 mm for a period of six days. Surfactant produced by *P. aeruginosa* also showed good oil clearing zone of 12 mm. However, *Bacillus pumilus* had the lowest zone of clearance showing that the concentration of surfactant produced by this isolate was very low.

The findings in this study revealed that there was an increase in the optical density of the growth medium indicating the growth of the test organism. In previous studies, researchers have used the measurement of optical density as an index for the determination of the growth of microorganisms (Janek *et al*., 2013; Mariashobana *et al*., 2014). The result also showed reduction in surface tension of the culture medium after 10 h suggesting the production of biosurfactant by *P. aeruginosa* HB6 (39). Surface tension measurement is useful for determining surfactant production. Biosurfactants with lesser molecular weight reduces surface tension (Naughton *et al*., 2019). The increase in bacterial growth also accounted for the decrease in surface tension, which indicates that the rate of biosurfactant production increases with the increase in the bacterial biomass. This corroborates the study of Raza *et al*. (2007) who documented that the production of rhamnolipids by *P. aeruginosa* mutant was associated with the growth of the microorganism. The lowest surface tension value of 27.7 mN/m was observed at 22 h incubation period. This is contrary to the results obtained by other researchers who reported that the lowest surface tension which corresponded to optimum biosurfactant production was obtained much later at 96 h (Patil *et al*., 2014; Agarry *et al*., 2015). Saikia *et al*. (2012), observed that optimum biosurfactants production occurred at 48 h. The production of biosurfactant within a short time range may be due to the spontaneous growth rate of this isolate. The low surface tension value of the culture medium suggest that the quality of the surfactant produced. Santos *et al*. (2016) documented that good surfactants with excellent surface activity can decrease water surface tension from 72 to 35 mN/m.

The microbial surfactants produced by *Pseudomonas* aeruginosa HB6 (39) were characterized as mono and di-rhamnolipids. This corroborates other reports showing the production of mono and di-rhamnolipids by *P. aeruginosa* (Samadi *et al*., 2012; Irfan- Maqsood & Seddiq-Shamma, 2014). Biosurfactant produced by *P. aeruginosa* HB6 (39) had a little higher percentage of mono-rhamnolipids (48.63%) to di-rhamnolipids (44.53%). It is noteworthy that most studies previously reported in the literatures revealed the occurrence of higher percentage of di-rhamnolipids congener in the rhamnolipids produced by *P. aeruginosa*, making the result obtained in this study very interesting. Several factors including bacterial strain used, type of carbon substrate, age of culture and cultural conditions affects the rhamnolipids congener distribution (Bharali & Konwar, 2011). The type of gene present in the isolates used for the production of rhamnolipids may be responsible for the variation in the ratio of mono-rhamnolipids and di-rhamnolipids (Zhou *et al*., 2019).

V. Conclusion

Pseudomonas aeruginosa HB6 (39) obtained from petroleum contaminated soils produced rhamnolipids with mono-rhamnolipids and di-rhamnolipids congeners. This further confirmed that more biosurfactant-producing strains are yet to be identified, hence increased effort in the search for biosurfactant producers as well as optimization of various factors affecting biosurfactant production could lead to the discovery of more strains with better yielding capacity. Industrial production of rhamnolipids with excellent surface activity using *Pseudomonas aeruginosa* HB6 (39) would

reduce dependency on chemical surfactant used for remediation of contaminated environment.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Abalos A, Pinazo A., Infante M. R., Casals M., García F., & Manresa A. (2001). Physicochemical and antimicrobial properties of new rhamnolipids produced by *Pseudomonas aeruginosa* AT10 from soybean oil refinery wastes. *Langmuir*, 17**,** 1367-1371.

Abdel-Mawgoud A., Lepine F., & Deziel E. (2010). Rhamnolipids: Diversity of structures, microbial origins and roles. *Applied Microbiology and Biotechnology*, 86, 1323-1336.

Abouseoud M., Maachi R., Amrane A., Boudergua S., & Nabi A. (2008). Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens. Desalination*, 223, 143-151.

Agarry S. E., Salam K. K., Arinkoola A. & Aremu, O. M. (2015). Biosurfactant production by Indigenous *Pseudomonas* and *Bacillus* species isolated from Auto-Mechanic Soil Environment: Towards Microbial Enhanced Oil Recovery. *European Journal of Engineering and Technology*, 3(6), 27-39.

Agyeman-Duah E., Mak-Mansah E., & Mills-Robertson F. C. (2017). Hydrocarbon Degrading Bacteria in Petroleum-Contaminated Soil at Suame Magazine. *Journal of Earth Sciences and Environmental Studies*. 2(3), 2018-2026.

Ajayi, A. O., & Abiola, A. K (2018). Microbial Diversity of Petroleum Polluted Soil at Ayetoro Community in Ilaje Riverine Oil Producing Areas of Ondo State, Nigeria. *Progress in Petroleum Science*, 1(5), 122-128.

Banat I. M., Carboué Q., Saucedo-Castañeda G., & Cázares-Marinero, J. J. (2021). Biosurfactants: The Green Generation of Speciality Chemicals and Potential Production Using Solid-State Fermentation (SSF) Technology. *Bioresour Technol* 320 part A 124222.

Banat I. M., Franzetti A., Gandolfi I., Bestetti G., Martinotti M. G., Fracchia, L., *et al*. (2010). Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology*, 87 (2), 427-444.

Baskaran S. M., Zakaria M. R., Mukhlis A. S., Sabri A., Mohamed M. S., Wasoh H., *et al*. (2020). Valorization of biodiesel side stream waste glycerol for rhamnolipids production by *Pseudomonas aeruginosa* RS6. *Environ Pollution*, 276,

Bento F. M., Camargo F., de Oliveira A., Okeke B. C., & Frankenberger, W. T. (2005). Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. *Microbiological Research,* 160, 249-255.

Beulah, B. F. P. (2018). Screening and extraction of biosurfactant bacteria from oil contaminated soils. *International Journal of Advanced Research*, 6(2), 1873-1878.

Bharali, P., & Konowar, B. K. (2011). Production and physicochemical characterization of a biosurfactant produced by *Pseudomonas aeruginosa* OBP1 isolated from petroleum sludge. *Applied Biochemistry and Biotechnology*. 164, 1444-1460.

Bhattacharya B., Ghosh T. K. & Das N. (2017). Application of Bio-Surfactants in Cosmetics and Pharmaceutical Industry. *Scholars Academic Journal Pharmacy*, 6(7), 320-329.

Bodour, A. A., & Miller-Maier, R. M. (1998). Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *Journal of Microbiological Methods*, 32, 273-280.

Bodour A. A., Dress K. P., & Maier R. M. (2003). Distribution of biosurfactant-producing bacteria in undisturbed and contaminated arid southwestern soils. *Applied Environmental Microbiology,* 69, 3280-3287.

Chebbi A., Elshikh M., Haque F., Ahmed S., Dobbin S., Marchant R., *et al*. (2017). Rhamnolipids from *Pseudomonas aeruginosa* strain W10; as antibiofilm/antibiofouling products for metal protection. *Journal of Basic Microbiol*ogy 9999, 1-12.

Chikere C. B., Okpokwasili G. C., Chikere B. O. (2009). Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. *African Journal of Biotechnology*, 8(11), 2535-2540

Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnology Research International*, 9, 18-23.

Das P., Yang X. P., & Ma L. Z. (2014). Analysis of biosurfactants from industrially viable Pseudomonas strain isolated from crude oil suggests how rhamnolipids congeners affect emulsification property and antimicrobial activity. Frontier *Microbiology*, 5, 696. doi: 10.3389/fmicb.2014.00696

Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Review*, 61(1), 47-64.

Enaigbe A. A., Imade O. S., Akpoka O. A., Ufuah E., Izevbuwa O. E., & Okhuebor, S.O. (2020). Characterization of authocthonous bacterial isolates with the potentiality to degrade spent engine oil from contaminated soil area enriched with glycine max. *Journal of Applied Science and Environmental Management*. 24(8), 1463-1466.

Fadhile A., Abu-Hassan H., Idris M., Sheikh-Abdullahi S. R., Anuar N. & Tibin E. M. (2016). Biosurfactant production by hydrocarbon-degrading bacteria (HDB) *Serratia mercescens*: optimization using central composite design (CCD). *Journal of Industrial and Engineering Chemistry,* 3201, 1-9.

Hamzah A., Sabturani N., & Radiman S. (2013). Screening and optimization of biosurfactant production by the hydrocarbon-degrading bacteria. *Sains Malaysiana*, 42(5), 615-623.

Hanano A., Shaban M., & Almousally I. (2017). Biochemical, molecular, and transcriptional highlights of the biosynthesis of an effective biosurfactant produced by *Bacillus safensis* pha3, a petroleum-dwelling bacteria. *Frontier Microbiology,* 8(77), 1-17.

Irfan-Maqsood, M., & Seddiq-Shamma, M. (2014). Rhamnolipids- Well-Characterized Glycolipids with potential Broad Applicability as Biosurfactants. *Industrial Biotechnology*, 285-291.

Irorere V. U., Tripathi L., Marchant R., McClean S., & Banat I. M. (2017). Microbial rhamnolipid production: a critical re-evaluation of published data and suggested future publication criteria. *Applied Microbiology and Biotechnology*, 101, 3941-3951.

Irving A. M., Andersen G. L., Baltz D. M., Caffey R. H., Carman K. R., Fleeger J. W., *et al*. (2012). Oil impacts on coastal wetlands: Implications for the Mississippi River Delta ecosystem after the deepwater horizon oil spill. *BioScience Journal*, 6, 562-574.

Jain D. K., Collins-Thompson D. L., Lee H., & Trevors J. T. (1991). A drop-collapsing test for screening surfactant-producing microorganisms. *Journal of Microbiological Methods*, 271-279.

Janek T., Marcin L., & Anna. (2013). Identification and characterization of biosurfactants produced by the Arctic bacterium *Pseudomonas putida* BD2. Colloids and surfaces B: *Biointerfaces*, 110, 379- 386.

Karanth N. G. K., Deo P. G., & Veenandig N. K. (1999). Microbial production of biosurfactants and their importance. *Journal of Fermentation Technology,* 77, 116-126.

Lima T. M., Procopio L. C., Brandao F. D., Carvalho A. M., Totola M. R. & Borges A. C. (2011). Biodegradability of bacterial Surfactants. *Biodegradation*, 22(3), 585-592.

Marchant, R., & Banat, I. M. (2012). Biosurfactants: a sustainable replacement for chemical surfactants? *Biotechnology Letters,* 34, 1597-1605**.**

Mariashobana A., Nalini K., & Thangapadian, V. (2014). Studies on Biosurfactant produced by Hydrocarbon Degrading Bacteria. *Journal of Advanced Botany and Zoology*, 2(2), 1-6.

Morikawa M., Hirata Y., & Imanaka T. (2000). A study on the structure-function relationship of lipopeptide biosurfactants. *Biochemistry and Biophysics Acta*, 1488(3), 211-218

Moussa T. A. A., Mohamed M. S., & Samak N. (2014). Production and characterization of Di-rhamnolipid produced by pseudomonas aeruginosa TMN. *Brazilian Journal of Chemical Engineering*. 31(4), 867-880.

Muhling M., Woolven-Allen J., Murrell C., & Joint I. (2008). Improved group-specific PCR primers for denaturing gradient gel electrophoresis analysis of the genetic diversity of complex microbial communities, *ISME Journal*, 2, 379-392.

Mulligan, N. C. (2005). Environmental application for biosurfactants. *Environmental Pollution*, 133, 183-198.

Naughton P. J., Marchant R., Naughton V., & Banat I. M. (2019). Microbial Biosurfactants: Current trends and applications in Agricultural and Biomedical industries. *Journal of Applied Microbiology*, 127(1), 12-28.

Ndibe T. O., Eugene W. C., & Usman J. J. (2018). Screening of Biosurfactant-Producing Bacteria Isolated from River Rido, Kaduna, Nigeria. *Journal of Applied Science and Environmental Management*, 22(11), 1855-1861.

Nkem B. M., Halimoon N., Yusoff F., & Wan J. W. (2019). Isolation and Optimization of Diesel-Oil Biodegradation using *Cellulosimicrobium cellulans* from Tarball *Pertanika Journal of Science and Technology*, 27(3), 1031-1040.

Odokuma L. O., & Dickson A. A. (2009). Bioremediation of a crude oil polluted tropical mangrove environment. *Journal of Applied Science and Environmental Management,* 7, 23-29.

Onifade, A. K. & Abubakar, F. A. (2007) Characterization of Hydrocarbon-Degrading Microorganisms Isolated from Crude Oil Contaminated Soil and Remediation of the Soil by Enhanced Natural Attenuation. *Research Journal of Biological Sciences*, 2, 36-40.

Patil S., Anuradha P., & Aruna, K. (2014). Studies on optimization of biosurfactant production by *Pseudomonas aeruginosa* F23 isolated from oil contaminated soil sample. *International Journal of Current Biotechnology*, 2(4), 20-30.

Perfumo A., Rudden M., Smyth T. J. P., Marchant R., Stevenson P. S., Parry N. J., *et al*. (2013). Rhamnolipids are conserved biosurfactants molecules: implications for their biotechnological potential. *Applied Microbiology and Biotechnology,* 97(16), 7297-7306.

Pradnya, A. J., & Dhiraj, B. S. (2014). Screening and isolation of biosurfactant producing bacteria from petroleum contaminated soil. *European Journal of Experimental Biology*, 4(4), 164-169.

Ramdass, A. C., & Rampersad, S. N. (2021). Diversity and oil degradation potential of culturable microbes isolated from chronically contaminated soils in Trinidad. *Microorganisms*, 9(1167), 1-29.

Raza Z. M. A., Rehman A., Khan M. S., & Khalid, Z. M. (2007). Improved production of biosurfactant by a *Pseudomonas aeruginosa* mutant using vegetable oil refinery wastes, *Biodegradation*, 18, 115-121.

Rodriguez-Lopez L., Rincon-Fontan M., Venico X., Moldes A.B., & Cruz J. M. (2019). Biodegradability study of the biosurfactants contained in a crude biosurfactants obtained from Corn Steep water. *Journal of Surfactants Detergents*, 23(1), 1-12.

Saikia R., Deka S., Deka M., & Banat I. M. (2012). Isolation of biosurfactant-producing *Pseudomonas aeruginosa* RS29 from oil-contaminated soil and evaluation of different nitrogen sources in biosurfactant production. *Anal Microbiology*, 62, 753-763.

Samadi N., Abadian N., Ahmadkhaniha R., Amini F., Dalili D., Rastkari N., *et al*. (2012). Structural characterization and surface activities of biogenic rhamnolipid surfactants from Pseudomonas aeruginosa isolate MN1 and synergistic effects against methicillin-resistant Staphylococcus aureus. *Folia Microbiology*, 57, 501-508.

Santos D. K., Rufino R. D., Luna J. M., Santos V. A., & Sarubbo L. A. (2016). Biosurfactants: multifunctional Biomolecules of the 21st Century. *International Journal of Molecular Science*, 17(3), 401.

Sekhon-Randhawa, K., & Rahman, P. K. S. M. (2014). Rhamnolipid biosurfactants-past, present, and future scenario of global market, *Frontier Microbiology*, 5, 454.

Shaieb, F. M., Elghazawani, A. H., & Issa, A. (2015). Studies on crude oil degrading bacteria isolated from the Libyan Desert. *International Journal of Current Microbiology and Applied Sciences*, 4(2), 920-927.

Shreve, G. S., & Makula, R. (2019). Characterization of a new rhamnolipid biosurfactant complex from *Pseudomonas* isolate DYNA270. *Biomolecules*, 9, 885

Siddhartha G., Nitschke M., Haddad R., Eberlin M. N., & Contiero J. (2006). Production of *Pseudomonas aeruginosa* LBI rhamnolipids following growth on Brazillian native oils. *Process Biochemistry,* 41, 483-488.

Silva M. A. M., Silva A. F., Rufino R. D., Luna J. M., Santos V. A., & Sarubbo V. (2017). Production of Biosurfactants by *Pseudomonas* Species for Application in the Petroleum Industry. *Water and Environmental Research*, 89, 117

Sinumvay, J. P., & Ishimwe, N. (2015). Agriculture and Food Applications of Rhamnolipids and its production by *Pseudomonas aeruginosa. Journal of Chemical Engineering and Process Technology*, 6(223), 1-8.

Subathra M. K., Immanuel G., & Suresh A. H. (2013). Isolation and Identification of hydrocarbon degrading bacteria from Ennore creek. *Bioinformation*, 9(3), 150-157.

Toribio J., Escalante A. E., & Soberon-Chavez, G. (2010). Rhamnolipids production from bacteria other than *Pseudomonas aeruginosa*. *European Journal of Lipids Science Techniques*, 112(10), 1082-1087

Twigg M. S, Tripathy L., Zompra A., Salek K., Irorere V. U., Gutieirrez T., *et al*. (2018). Identification and characterization of short chain rhamnolipid production in a previously uninvestigated, non-pathogenic marine *Pseudomonad*. *Applied Microbiology and Biotechnology*, 102, 8537-8549

Udotong I. R., Eduok S. I., Essien J. P., Ita B. N. (2008). Density of hydrocarbonoclastic bacteria and polycyclic aromatic hydrocarbons accumulation in Iko River Mangrove ecosystem, Nigeria. Proceedings of World Academy of Science, *Engineering Technology*, 34, 830-836.

Urhibo, V. O., & Ejechi, B. O. (2017). Crude oil degradation potential of bacteria isolated from oil polluted soil and animal wastes in soil amended with animal wastes. *AIMS Environmental Science*, 4(2), 277-286.

Vijayakumar, S., & Saravanan, V. (2015). Biosurfactants- Types, sources and applications. *Research Journal of Microbiology*, 10, 181-192.

Voulgaridou G. P., Mantso T., Anestopoulos I., Klavaris A., Katzastra C., Kiousi D. E., *et al*. (2021). Toxicity Profiling of Biosurfactants Produced by Novel Marine Bacterial Strains. *International Journal of Molecular Sciences*, 22, 2383.

Zhou J., Xue R., Liu S., Xu N., Xin F., Zhang W., *et al*. (2019). High Di-rhamnolipid Production Using Pseudomonas aeruginosa KT1115, Separation of Mono/Di-rhamnolipids, and Evaluation of Their Properties. *Frontier Bioengineering and biotechnology*. 7, 245.

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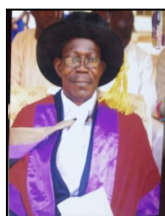

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