ISSN No:-2456-2165

Assessment and Characterization of Bacteria for Biosurfactant-Producing Ability from Lambda-Cyhalothrin-Contaminated Cultivated Soil in Moro, Kwara State, Nigeria

Zainab Biola Abdulsalam[1]*, Kehinde Imisi Temitope Eniola[2] and Sunday Awe[1]

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, PMB 1530, Ilorin, Kwara State, Nigeria
[2]Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, PMB 5006, Ilesha, Osun State, Nigeria
*Corresponding author: Zainab Biola Abdulsalam

Abstract:- Microorganisms produced biosurfactants are promising agents in the bioremediation of pesticide polluted soils as a result of their biodegradability and non-toxic nature. This study was aimed at screening bacteria for biosurfactant production in pesticide contaminated agricultural soil, and identify them using molecular technique. Ten bacteria species with potentials to degrade lambda-cyhalothrin isolated in our previous study were screened for biosurfactant production via the drop collapse, oil spreading, emulsification index, hydrocarbon overlay agar and bacterial adhesion to hydrocarbon tests. The biosurfactant producers were identified using the 16S rRNA. Results from the biosurfactant screening showed that out of the ten bacteria screened only four of the ten bacteria screened which were identified as *Bacillus* strains were strongly positive for production of biosurfactant. The four Biosurfactant-Producing Lambda Cyhalothrin-Degrading Bacteria (LCDB-BP) were identified using 16S rRNA as *Bacillus tequilensis* strain LCDB-BP1, *Bacillus subtilis* strain LCDB-BP2, *Bacillus altitudinis* strain LCDB-BP3, and *Bacillus subtilis* strain LCDB-BP4 with the accession numbers OP703607, OP703608, OP703609 and OP703610 respectively. These organisms can be useful for biosurfactant production and bioremediation of synthetic pyrethroid contaminated soil.

***Keywords**:- Biosurfactant, Synthetic Pyrethroids (SP), Lambda-cyhalothrin (LC), Bacillus species, cultivated soil.*

I. INTRODUCTION

Microorganisms like bacteria, fungi and yeast secrete biosurfactants, which are surface active metabolites, which either adhere to the cell surfaces or are excreted extracellularly into the growth medium [1]. They are amphiphilic agent with potentials for reduction of surface and interfacial tensions [2]. Biosurfactants constitute different compounds such as fatty acids, glycolipids, phospholipids, polymeric structures, neutral lipids, lipopeptidesand lipopolysaccharides [3-4]. These biomolecules have various applications as biocontrol agents in agriculture, health, cosmetic industries, pharmaceutical and food industries [5-6]. Montero-Rodríguez *et al.* [7] showed that biosurfactant have comparative advantages over synthetic surfactants in terms of reduced toxicity, increased biodegrading ability, specificity and ability to function at extreme pH, temperature and salinity conditions. Most of the reported biosurfactant producers are members of the genera *Achromobacter, Acinetobacter, Aeromonas, Alkaligenes, Arthrobacter, Bacillus, Corynebacterium, Flavobacterium, Klebsiella, Micrococcus, Moraxella, Proteobacteria, Pseudomonas and Streptococcus* [8-9].

Bacillus species are an interesting group of bacteria because of their broad distribution in the environment, biochemical versatility, and their effectiveness in protein production in the industries [10]. The most efficient producers of biosurfactants ever reported are the genus *Bacillus*, which have been isolated from different pesticide contaminated agricultural soils [11]. Some reports have shown that biosurfactant-producing bacteria also possess pesticide degrading abilities. Thus, the aim of the present study was to screen and identify biosurfactant-producing bacterial isolates from LC-contaminated agricultural soil.

II. MATERIALS AND METHODS

A. *Chemicals*

All chemicals and reagents used in this study were of analytical grade and were bought from the Central Research and Diagnostic Laboratory, Ilorin, Kwara State, Nigeria.

B. *Subculturing of LC-Degrading Bacteria*

Pure cultures of ten LC-Degrading bacterial isolates from our previous study were subcultured on nutrient agar and tested for viability [12]. Broth culture of the isolates were then prepared in nutrient broth and standardized to McFarland 0.5. For this study the standardized cultures were used.

C. Screening for Potential Biosurfactant Producers

D. Preparation of Cell Free Supernatant (CFS)

Each of the isolates was inoculated into 100 ml mineral salt medium enriched with LC in separate 500 ml Erlenmeyer flask. The flasks were kept in incubator shaker at 37 ºC for 7 days at 160 rpm. After 7 days of incubation, CFS were prepared from the culture broths. Broths from each flask were spinned in the centrifuge at 6000 revolution per minute for 15 minutes, then the supernatants were filtered using a filter paper of 0.45 µm pore size. The CFS was used to carry out the screening tests, all of which were performed in triplicates [13].

E. Drop Collapse Test (DC)

The DC test was done using the methods of [14-15]. Crude oil (10 µl) was used to coat the wells of a micro titer plate thinly and then left undisturbed for 1 hour at 37 ˚C to form uniform thin coats of the oil in the well. Then 10 µl of the CFS was added to the center of each well. The collapse of the CFS droplets within 1-2 minutes shows that biosurfactant is present, while no change in the shape of the droplet shows absence of biosurfactant. A chemical surfactant (Triton X-100) was used as the positive control while de-ionized water was used as the negative control.

F. Hydrocarbon Overlay Agar Test (HOA)

The HOA test was done by coating mineral salt agar plates with 50 µl of crude oil. The pure bacteria were spotted on the coated plates and incubated at 30 ºC for 7 days. Colonies surrounded by emulsified halos were considered positive for biosurfactant production, while colourless colonies indicate absence of biosurfactant production by the isolates [16].

G. Oil Displacement Assay (OD)

The OD test was done by adding 40 ml of distilled water in to a plastic Petri dish. Then 10 ml of crude oil was added to the surface of the water. Finally, 10 ml of CFS was gently added to the oil-water surface [17]. The displacement of oil and the formation of a clear zone indictes biosurfactant production in the CFS. The diameter of clear zone was measured using a meter rule. Distilled water without surfactant was used as the negative control, while Triton X-100 was used as the positive control [18].

H. Bacterial Adhesion to Hydrocarbons (BATH) Assay

BATH assay was carried to determine the hydrophobicity of the cell surface. The CFS (2 mL) with 100 µL of crude oil were mixed and vortexed shaken for 3 min in test tubes (10 × 100 mm). After shaking, the hydrocarbon and aqueous phase was left to separate for 1 hour. Cell surface hydrophobicity was expressed as cell adherence to crude oil and was calculated using:

Bacterial adherence (%) = 1 – (initial OD without shaking /OD of vortexed suspension) × 100 [19].

I. Emulsification Index (EI)

The emulsifying capacity of the four LCDB-BP isolates were estimated using the EI test. Crude oil (1.5 ml) was mixed with 1.5 ml of CFS in a test tube. It was then vortexed-shaken at high speed for 2 min., and left undisturbed for 24 hours. The emulsification index percentage was calculated using the equation: EI = (Height of emulsion formed / Height of solution) × 100 [14].

J. Molecular Identification of the Biosurfactant-Producers

After the screening tests, the four potential biosurfactants producers; LCDB1, LCDB2, LCDB3 and LCDB4 were selected for molecular characterization using 16S rDNA gene sequencing homology. The DNA of the four isolates were extracted using the procedures of Presto™ Mini g DNA bacteria kit from the (Geneaid) company. The amplification (polymerase chain reaction) of the extracted DNA were done using the universal primers 27F (5-AGAGTTTGATCCTGGCTCAG3) and 1492R (5-GGTTACCTTGTTACGACTT-3), making a total volume of 50 µl. The initial denaturation step was done at 96 °C for 3 min. This was followed by 27 cycles of 96 °C for 30 seconds. Then annealing was done at a temperature of 56 °C for 25 seconds, followed by extension at 72 °C for 15 seconds and then, final extension at 72 °C for 10 minutes [20]. The PCR products separated based on molecular weight using a 1% (w/v) agarose gel made with TBE buffer. The DNA was viewed under UV light using ethidium bromide DNA stain. Then, the amplified DNA was purified and sequenced. The nucleotide sequences were determined at Inqaba Biotec, South Africa. The resulting 16S rRNA gene sequences were corrected and compared with nucleotide sequences of NCBI using BLAST tools to estimate the sequence homology and identify the isolates. The multiple sequence alignment was carried out using CLUSTAL Omega “https://www.ebi.ac.uk/Tools/msa/clustalo/“. The MEGA version 7.0 was used to construct the phylogenetic tree and the nucleotide sequences were submitted to the NCBI GenBank database for allocation of accession numbers [20].

III. RESULTS

Ten (10) bacterial isolates capable of degrading LC that have been isolated and characterized in our previous study were screened for production of biosurfactant (Table 1).The ten isolates named as LCDB1-LCDB10 were analyzed for biosurfactant production and only four isolates LCDB 1-4 were positive for DC, HOA and OD tests. The biosurfactant-producing abilities of the four positive isolates were further confirmed using the BATH and EI tests which ranged from 53-65 % and 62-70 % respectively. While the isolates LCDB5-LCDB10 were negative for DC, HOA and OD tests and also have low values for the BATH and EI tests which ranged from 15-25 % and 17-25 % respectively (Table 1).

Table 1: Screening of LCDB Isolates for Biosurfactant Production

Isolates	DC	HOA	OD (cm)	BATH (%)	EI (%)
LCDB1	+	+	**12.80**	**60**	**68**
LCDB2	+	+	**08.35**	**53**	**62**
LCDB3	+	+	**14.30**	**65**	**70**
LCDB4	+	+	**10.12**	**57**	**65**
LCDB5	-	-	**00.00**	**15**	**20**
LCDB6	-	-	**00.00**	**20**	**17**
LCDB7	-	-	**00.00**	**23**	**20**
LCDB8	-	-	**00.00**	**21**	**25**
LCDB9	-	-	**00.00**	**25**	**23**
LCDB10	-	-	**00.00**	**20**	**21**

Key: DC: Drop Collapse; HOA: Hydrocarbon Overlay Agar; OD: Oil Displacement; BATH: Bacterial Adhesion to Hydrocarbon; EI: Emulsification Index + =Positive; - = Negative

The results obtained from the analysis of the 16S rRNA gene sequences revealed that the biosurfactant-producing LC-degrading bacterial isolates (LCDB1, LCDB2, LCDB3 and LCDB4) were *Bacillus tequilensis* strain LCDB-BP1, *Bacillus subtilis* strain LCDB-BP2, *Bacillus altitudinis* strain LCDB-BP3 and *Bacillus subtilis* strain LCDB-BP4 respectively (Fig. 1). The phylogenetic tree of the four LCDB-BP isolates was constructed (Fig. 1).

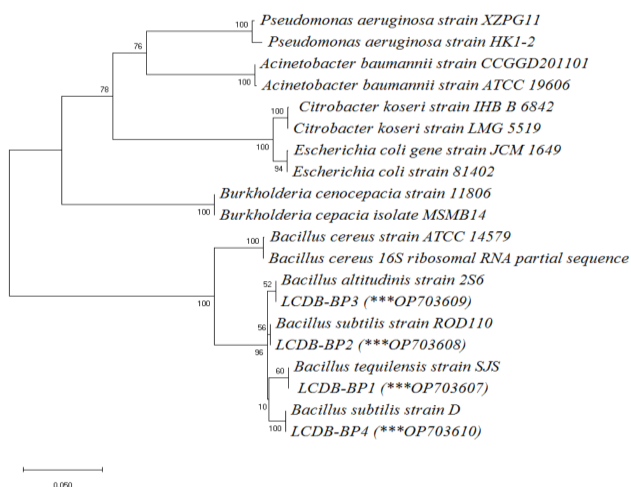

Fig. 1: Phylogenetic tree of the four LCDB-BP isolates constructed using the Neighbor-Joining method (http://www.nlm.nih.gov/BLAST).

IV. DISCUSSION

The positive collapse test is an indication of the presence of surface active substance [21] in the CFS from the isolates, which can be taken as the evidence of production of biosurfactant by the isolates. Shoeb *et al.* [16] reported similar observation with *Bacillus* species. The capability of the four isolates (LCDB1, LCDB2, LCDB3 and LCDB4) as biosurfactant producing is further demonstrated in the HOA test by the formation of blue halos around their colonies while the colonies of other six isolates remain colourless. The formation of blue halos around colonies is an indication of biosurfactant production [22]. Nayarisseri *et al.* [19] reported blue halos surrounding the colonies of a biosurfactant producing *Bacillus* species. Similarly, El-Gebaly [23] reported that 40% of the *Bacillus* species isolated from soil had blue halos around their colonies. Sohail and Jamil [24] also reported halos around the colonies of all biosurfactant producing isolates.

In this study the four strains gave zone diameters (08.35-14.30 cm) with crude oil. This is in line with the 8.0 cm diameter observed in a *Bacillus* species reported by Nawazish *et al.* [25], and 10.00-14.20 cm reported by Al Yousif *et al.* [17]. The isolate LCDB3 can thus be consider a better producer of biosurfactant than the other because it gave the highest displacement zone diameter (14.30 cm), followed by LCDB1 (12.80 cm), LCDB4 (10.12cm), LCDB2 (08.35cm) while no oil displacement was seen in the other six isolates. According to [26], the zone diameter is directly proportional to the concentration and activity of biosurfactant. The OD test is more sensitive than other methods of identifying biosurfactant producers and more suitable since it can detect low levels of biosurfactant production [27]. However, the diameters were smaller than 31.20 cm which Abubakar *et al.* [28] observed in *Bacillus* species, but higher than 4.90 cm obtained for *Bacillus subtilis* by Umar *et al.* [29] and 9.22-9.65 cm obtained for different *Bacillus* species by Ilusanya *et al.* [30].

The results obtained from the BATH test, also known as cell hydrophobicity test showed that the four isolates LCDB1-LCDB4 produced biosurfactant (53-65%), while isolates LCDB5-LCDB10 (15-25%) did not. The results obtained in this study agree with the findings of [30] who reported a BATH value of 50% for a biosurfactant producing *Pseudomonas aeruginosa* isolated from marine sediment. These results also agree with the reports of Nayarisseri *et al.* [32] who demonstrated a value BATH of 60% of by biosurfactant producing *Bacillus tequilensis* ANSKLABO4 strain isolated from brackish river water. According to [20], the ability of bacteria to adhere to hydrocarbons is a characteristic feature of biosurfactant-production of microorganisms. Biosurfactant production is an important survival strategy for microorganisms because it facilitates their attachment, and adhesion to natural substrates [34]. The formation of stable emulsions with crude oilby the four isolates LCDB1, LCDB2, LCDB3 and LCDB4 further affirms that the isolates were biosurfactant producers. The isolate LCDB3 (70.0%) can be regarded as the best biosurfactant producer among the isolated strains, with the order of performance as: LCDB1 (68.0%)>LCDB 4(65.0%)> LCDB 2 (62.0%).

The results of the emulsification index study in this study is like the findings of Nawazish *et al.* [25], who reported an emulsification index of 66.4 % for *Bacillus* species with crude oil. Similar emulsification indices for crude oil by *Bacillus subtilis* have been reported 63.0 % by Shafiei *et al.* [34], 64.0 % by Parthipan *et al.* [35], 58.0 % by

Adamu and Ibrahim [36] and 56.0 % by Wu *et al*. [37]. Patowary *et al.* [38] reported a maximum emulsification activity of 100%. The results of emulsification index revealed variations in the emulsification potentials of the isolates in this study and the other studies. The implication this variation is that these isolates possess different degrees of biosurfactant activity. According to Aparna *et al*. [39] biosurfactants activity are species- specific. Gupte and Sonawdekar [40] had reported that a strain of *Bacillus cereus* showed a high emulsification ability and thus have a valuable role in the bioremediation of contaminated environments. The screening parameters used in this study were consistent with previous reports by [19, 41-42]. According to Kiran *et al*. [43], a combination of methods are recommended for the identification of all types of biosurfactants, since a single method may not give accurate result.

The four potential biosurfactant producers: LCDB-BP1, LCDB-BP2, LCDB-BP3, and LCDB-BP4 were identified as strains of *Bacillus* species. These are: *B.tequilensis* strain LCDB-BP1, *B.* subtilis strain LCDB-BP2, *B. altitudinis* strain LCDB-BP3 and *B. subtilis* strain LCDB-BP4 with 99, 95, 95 and 99 % sequence relatedness to *B.tequilensis* strain SJS, *B. subtilis* strain ROD110, *B. altitudinis* strain 2S6 and *B. subtilis* strain D respectively (Fig. 1). The obtained sequences were submitted to the Genbank with the accession numbers OP703607, OP703608, OP703609 and OP703610. The phylogenetic tree analysis placed the four *Bacillus* isolates into four subgroups. The first sub-group included *Bacillus altitudinis*. The second sub-group comprised *B. subtilis* strain 2S6, while the third and fourth sub-groups consisted of *B. tequilensis* and *B. subtilis* strain D respectively. These results are consistent with the findings of [36-37, 44-45] who had identified different *Bacillus* species as biosurfactant producers. The strains of *Bacillus subtilis and Bacillus tequilensis* identified in this study have been reported as biosurfactant producers in several studies [32, 46, 17, 24, 47, 29]. In the present study, the ability of the isolates to produce biosurfactant suggests that, they can be used as emulsifying agents for many industrial applications. This also allow their direct applications in environmental remediation of contaminated soil.

V. CONCLUSION

The four *Bacillus* species with potentials to produce biosurfactant were identified in this study. Thus, these isolates have valuable role in the bioremediation of contaminated environments. They can also be applied for commercial production of biosurfactants which can be useful in various environmental applications.

REFERENCES

[1]. N. D. Ortega-de la Rosa, J. L. Va´zquez-Va´zquez, S. Huerta-Ochoa, M. Gimeno and M. Gutie´rrez-Rojas, Stable Bioemulsifiers Produced by *Acinetobacterbouvetii* UAM25 Growing in Different Carbon Sources. Bioprocess and Biosystems Engineering, 2018, vol. 41, pp. 859-869. https://doi.org/10.1007/s00449-018.1920-5

[2]. V. M. Jahanbani, A. E. Ganji, T. Deihimi, A. Niazi and S. Ayatollahi, S. Isolation and Screening of *Bacillus subtilis* MJ01 for MEOR application: Biosurfactant Characterization, Production Optimization and Wetting Effect on Carbonate Surfaces. Journal of Petroleum Exploration and Production Technology, 2019, vol. 9, no. 1, pp. 233- 245. https://doi.org/10.1007/s13202-018-0457-0

[3]. I. M. Banat,S. K. Satpute, S. S. Cameotra, R. Patil and N. V. Nyayanit, Cost Effective Technologies and Renewable Substrates for Biosurfactants Production. Frontiers in Microbiology, 2014, vol. 5, pp. 1–18. https://doi.org/10.3389/fmicb.4014.00697

[4]. T. Soltanighias, A. E. A. Singh, S. K. Satpute, A. G. Banpurkar, A. Koolivand and P. Rahi, Assessment of Biosurfactant-Producing Bacteria from Oil-Contaminated Soils and their Hydrocarbon Degradation Potential. Environmental Sustainability, 2019, vol. 2, pp. 1-12. https://doi.org/10.1007/s42398-019-00074-0

[5]. A. H. M. Resende, J. M. Farias, D. D. Silva, R. D. Rufino, J. M. Luna, T. C. M. Stamford and L. A. Sarubbo, Application of Biosurfactants and Chitosan in Toothpaste Formulation. Colloids and Surfaces B: Biointerfaces, 2019, vol. 181, pp. 77- 84 .https://doi.org/10.1016/j.colsurfb.2019.05.032

[6]. R. Fopase, S. R. Pathode, S. Sharma, P. Datta and L. M. Pandey, Lipopeptide and Essential Oil Based Nanoemulsion for Controlled Drug Delivery. Polymer-Plastics Technology and Materials, 2020, vol. 59, no. 18, pp. 2076-2086. https://doi.org/10.1080/25740881.2020.178

[7]. **D.** Montero-Rodríguez, R. de Souza –Mendonça, A. F. de Souza, I. N. da Silva- Ferreira, R. F. S. Andrade and G. M. Campos-Takaki, Solid-State Fermentation for Low-Cost Production of Biosurfactant by Promising *Mucorhiemalis* UCP 1309. Research, Society and Development, 2022, vol. 11, no. 6, pp. 28817-28826. https://doi.org/10.33448/rsd-v11i6.28817

[8]. Y. H. Hong, C. C. Ye, Q. Z. Zhou, X. Y. Wu, J. P. Yuan, J. Peng, H. Deng and J. H. Wang, Genome Sequencing Reveals the Potential of *Achromobacter* sp. HZ01 for Bioremediation. Frontiers in Microbiology, 2017, vol. 8, pp. 1–14. https://doi.org/10.3389/fmicb.2017.01507

[9]. M. S. Twigg, L. Tripathi, A. Zompra, K. Salek, V. U. Irorere, T. Gutierrez, G. A. Spyroulias, R. Marchant and I. M. Banat, Identification and Characterisation of Short Chain Rhamnolipid Production in a Previously Uninvestigated, Nonpathogenic Marine Pseudomonad. Applied Microbioiology and Biotechnology, 2018, vol. 102, pp. 8537-8549. https://doi.org/10.1099/acmi.ac2019.po0066

[10]. M. M. Stancu, Biosurfactant Production by a *Bacillus megaterium* Strain. Open Life Sciences, 2020, vol. 15, pp. 629–637. https://doi.org/10.1515/biol-2020-0068

[11]. S. Akbari, N. H. Abdurahman, R. M. Yunus, F. Fayaz and O. R. Alara, Biosurfactants: A New Frontier for Social and Environmental Safety: A Mini Review. Biotechnology Resources and Innovation, 2018, vol. 2, no. 1, pp. 81–90. https://doi.org/10.1016/j.bior.2018.09.001

[12]. Z. B. Abdulsalam, K. I. T. Eniola and S. Awe, Isolation, Characterization and Screening of Potential LambdaCyhalothrin-degrading Bacteria from Cultivated Soil in Moro, Kwara State, Nigeria, JOBIMB, 2023, vol. 11, no. 1, pp. 22-25 https://doi.org/10.54987/jobimb.v11i1.801

[13]. T. Femi-Ola, O. Oluwole, T. Olowomofe and H. Yakubu, Isolation and Screening of Biosurfactant Producing Bacteria from Soil Contaminated with Domestic Waste Water. British Journal of Environmental Science, 2015, vol. 3, pp. 58-63. www.eajournals.org

[14]. K. M. Barakat, S. W. Hassan and O. M. Darwesh, Biosurfactant Production by Haloalkaliphilic*Bacillus* Strains Isolated from Red Sea, Egypt. Egypt Journal of Aquatic Resources, 2017, vol. 43, no. 3, pp. 205-211. https://doi.org/10.1016/j.ejar.2017.09.001

[15]. O. R. Gamez, A. A. Rodriguez, J. V. Cadre and J. G. Cabrera-Gomez, Screening and Characterization of Biosurfactant Producing Bacteria Isolated from Contaminated Soils with Oily Wastes. Journal of Environmental Treatment Techniques, 2017, vol. 5, no. 1, pp. 5-11. ISSN: 2309-1185. https://www.jett.dormaj.com

[16]. E. Shoeb, N. Ahmed, J. Akhter, U. Badar, K. Siddiqui, F. A. Ansari, M. Waquar, S. Imtiaz, N. Akhtar, Q. Shaikh, R. Baig, S. Butt, S. Khan, S. Hussain, B. Ahmed and M. A. Ansari, Screening and Characterization of Biosurfactant Producing Bacteria Isolated from the Arabian Sea coast of Karachi. Turkish Journal of Biology, 2015, vol. 39, pp. 210-216. https://doi.org/10.3906/biy-1405-63

[17]. N. A. Alyousif, Y. Y. Y. A. Luaibi and W. Hussein, Distribution and Molecular Characterization of Biosurfactant-Producing Bacteria. Biodiversitas, 2020, vol. 21, pp. 4034-4040. https://doi.org/10.13057/biodiv/d210914

[18]. A. I. Adetunji and A. O. Olaniran, Production and Potential Biotechnological Applications of Microbial Surfactants: An Overview. Saudi Journal of Biological Sciences, 2021, vol. 28, pp. 669–679. https://doi.org/10.1016/j.sjbs.2020.10.58

[19]. A. Nayarisseri, P. Singh and S. K. Singh, Screening, Isolation and Characterization of Biosurfactant Producing *Bacillus subtilis* strain ANSKLAB03. Bioinformation, 2018, vol. 14, no. 6, pp. 304-314. https://doi.org/10.6026/97320630014304

[20]. S. Swaathy, V. Kavitha, A. S. Pravin, G. Sekaran, A. B. Mandal and A. Gnanamani, Phylogenetic Framework and Biosurfactant Gene Expression Analysis of Marine *Bacillus* spp. of Eastern Coastal Plain of Tamil Nadu. International Journal of Bacteriology, 2014, vol. 86, pp. 4-9. https://dx.doi.org/10.1155/2014/860491

[21]. N. A. K. Sima, A. Ebadi, N. Reiahisamani and B. Rasekh, Bio-Based Remediation of Petroleum Contaminated Saline Soils: Challenges, the Current State-of-the-Art and Future Prospects. Journal of Environmental Management, 2019, vol. 250, pp. 109476-109488. https://doi.org/10.1016/j.jenvman.2019.109476

[22]. K. Siddiqui, F. Ansari, Q. Shaikh, E. Shoeb, S. Khan, S. Hussain, J. Akhter, M. Ansari, S. Butt, N. Ahmed, R. Baig, S. Imtiaz, B. Ahmed, S. Khan, N. Akhtar, U. Badar and M. Waqar, Screening and Characterization of Biosurfactant-Producing Bacteria Isolated from the Arabian Sea Coast of Karachi 2. Turkish Journal of Biology, 2015, vol. 39, pp. 210–216. https://doi.org/10.3906/biy-1405-63

[23]. E. El-Gebaly, Screening of Biosurfactant Production by Bacterial Strains Isolated from Oil Contaminated Sites Near Gas Stations in Egypt. Al-Azhar Journal of Pharmaceutical Sciences, 2020, vol. 61, pp. 134-146. https://doi.org/10.21608/AJPS.2020-86023

[24]. R. Sohail and N. Jamil, Isolation of Biosurfactant Producing Bacteria from Potwar Oil Fields: Effect of Non-Fossil Fuel-Based Carbon Sources. Green Process Synthesis, 2020, vol. 9, pp. 77-86. https://doi.org/10.1515/gps-2020-0009

[25]. A. Nawazish, W. Fenghuan, X. Baocai, S. Bushra, U. Asad, N. Muhammad, W. Ce and T. R. Muhammad, Production and Application of Biosurfactant Produced by *Bacillus licheniformis* Ali5 in Enhanced Oil Recovery and Motor Oil Removal from Contaminated Sand. Molecules, 2019, vol. 24, pp. 4448-4466. https://doi.org/10.3390/molecules24244448

[26]. A. Roy, Effect of Various Culture Parameters on the Biosurfactant Production from Bacterial Isolates. Journal of Petroleum and Environmental Biotechnology, 2017, vol. 8, no. 6, pp. 350-361. https://doi.org/10.4172/2157-7463.1000350

[27]. A. A. Jayasree. and D. Latha, Isolation and Characterization of Biosurfactant Producing *Bacillus* sp. Journal of Pharmacy Research, 2018, vol. 12, no. 3, pp. 316-320. Corpus ID: 91723120. https://www.semanticscholar.org

[28]. U. Abubakar, U. B. Ibrahim, A. Y. Fardami, A. H. Kawo and S. M. Dankaka, Biosurfactant Production Potential of *Bacillus* Obtained from Dye Effluent. African Journal of Microbiology Research, 2020, vol. 14, no. 2, pp. 71-76. https://doi.org/10.5897/AJMR2019.9174

[29]. A. Umar, A. Zafar, H. Wali, M. P. Siddique, M. A. Qazi, A. H. Naeem, Z. A. Malik and S. Ahmed, Low-Cost Production and Application of Lipopeptide for Bioremediation and Plant Growth by *Bacillus subtilis* SNW3. AMB Express, 2021, vol. 11, pp. 165-186. https://doi.org/10.1186/s13568-021-01327-0

[30]. O. A. F. Ilusanya, A. J. Orjinta, I. B. Onajobi, F. M. Oyeyipo, E. A. Ogunsola and T. C. Fatunmbi, Screening for Biosurfactant Producing Bacteria from Petroleum Contaminated Soil of Automobile Workshops in Ago-Iwoye, Ogun State, Nigeria. Nigerian Journal of Microbiology, 2020, vol. 35, no. 1, pp. 5503 – 5511. www.nsmjournal.org.ng

[31]. R. Sumathi and N. Yogananth, Isolation and Identification of Biosurfactant Producing *Pseudomonas aeruginosa* from Marine Sediment Samples and its Antimicrobial Properties. International Journal of Advanced Research in Biological Sciences, 2016, vol. 3, no. 12, pp. 200-212. https://dx.doi.org/10.22192/ijarbs.2016.03.12.029

[32]. A. Nayarisseri, P. Singh and S. K. Singh, Screening, Isolation and Characterization of Biosurfactant-Producing *Bacillus tequilensis* strain ANSKLAB04 from Brackish River Water. International Journal of Environmental Science and Technology, Springer Nature, 2019, vol. 16, no. 11, pp. 7103-7112. https://doi.org/10.1007/s13762-018-2089-9

[33]. E. O. Fenibo, S. I. Douglas and H. O. Stanley, A Review on Microbial Surfactants: Production, Classifications, Properties and Characterization. Journal of Advanced Microbiology, 2019, vol. 18, pp. 1–22. https://doi.org/10.3390/microorganisms7110581

[34]. Z. Shafieia, W. M. Yusoff, A. Abdul Hamida, N. Moazamib, A. Hamzaha and T. Fooladi, Identification of Potential Local Isolated for Biosurfactant Production; AIP Conference Proceedings, 2013, vol. 1571, pp. 191-196. https://doi.org/10.1063/1.4858653

[35]. P. Parthipan, E. Preetham, L. L. Machuca, P. Rahman, K. Murugan and A. Rajasekar, Biosurfactant and Degradative Enzymes Mediated Crude Oil Degradation by Bacterium *Bacillus subtilis* A1. Frontiers in Microbiology,2017, vol. 8, pp. 193-206. https://doi.org/10.3389/fmicb.2017.00193

[36]. A. Adamu and D. B. Ibrahim, Biosurfactant Production by Bacteria Isolated from Local Tannery Effluent in Sokoto Metropolis. International Journal of Pure and Applied Science Research, 2021, vol. 12, no. 2, pp. 181-198. ISSN:2384-5918. www.arcnjournal.org

[37]. B. Wu, J. Xiu, L. Yu, L. Huang, L. Yi and Y. Ma, Biosurfactant Production by *Bacillus subtilis* SL and its Potential for Enhanced Oil Recovery in Low Permeability Reservoirs. Scientific Reports, 2022, vol. 12, pp. 7785- 7795. https://doi.org/10.1038/s41598-022-12025-7

[38]. K. Patowary, R. Patowary, M. C. Kalita and S. Deka, Characterization of Biosurfactant Produced During Degradation of Hydrocarbons Using Crude Oil as Sole Source of Carbon. Frontiers in Microbiology, 2017, vol. 8, pp. 279-292. https://doi.org/10.3389/fmicb.2017.00279

[39]. A. Aparna, G. Srinikethan and H. Smitha, Isolation, Screening and Production of Biosurfactant by *Bacillus Clausii* 5B. Research in Biotechnology, 2012, vol. 3, no. 2, pp. 49-56. ISSN:2229-791X. www.researchinbiotechnology.com

[40]. A. Gupte and S. Sonawdekar, Study of Oil Degrading Bacteria Isolated from Oil Contaminated Sites. IJRASET, 2015, vol. 3, pp. 345–349. ISSN:2321-9653. www.ijraset.com

[41]. C. G. Anaukwu, A. I. Ekwealor, C. C. Ezemba, V. N. Anakwenze, U. C. Okafor and E. J. Archibong, *Pseudomonas monteilii* and *Citrobactermurliniae,*Biosurfactant-Producing Bacteria Isolated from Nigerian Soil. British Microbiology Research Journal, 2015, vol. 10, no. 1, pp. 1-9. https://doi.org/10.9734/BMRJ/2015/19742

[42]. N. M. Sidkey, H. F. Mohamed and I. H. Elkhouly, Evaluation of Different Screening Methods for Biosurfactant Producers Isolated from Contaminated Egyptian Samples Grown on Industrial Olive Oil Processing Waste. British Microbiology Research Journal, 2016, vol. 17, np. 4, pp. 1-19. ISSN: 2231-0886. NLM ID: 101608140. www.sciencedomain.org

[43]. G. S. Kiran, T. A. Thomas and J. Selvin, Production of a New Glycolipid Biosurfactant from Marine *NocardiopsisLucentensis* MSA04 in Solid State Cultivation. Colloids, 2010, vol. 78, pp. 8–16. https://doi.org/10.1016/j.colsurfb.2010.01.028

[44]. G. J. Płaza, K. Chojniak, K. Rudnicka, P. Paraszkiewicz and P. Bernat, Detection of Biosurfactants in *Bacillus* Species: Genes and Products Identification. Journal of Applied Microbiology, 2017, vol. 34, no. 1, pp. 23-29. https://doi.org/10.1111/jam.12893

[45]. I. A. Phulpoto, Z. Yu, B. Hu, Y. Wang, F. Ndayisenga, J. Li, H. Liang and M. A. Qazi, Production and Characterization of Surfactin-like Biosurfactant Produced by Novel Strain *Bacillus nealsonii* S2MT and Its Potential for Oil Contaminated Soil Remediation. Microbial Cell Factories, 2020, vol. 19, pp. 145-157. https://doi.org/10.1186/s12934-020-01402-4

[46]. A. J. Das and R. Kumar, Production of Biosurfactant from Agro-Industrial Waste by *Bacillus safensis* J2 and Exploring its Oil Recovery Efficiency and Role in Restoration of Diesel Contaminated Soil. Environmental Technology and Innovation, 2019, vol. 16, pp. 100450. https://doi.org/10.1016/j.eti.2019.100450

[47]. A. Nayarisseri and S. K. Singh, Whole Genome Sequence of Biosurfactant Producing *Bacillus tequilensis*. Research Square, 2021, Vol. 1, pp. 1-58. https://doi.org/10.21203/rs.3.rs-115961/v2.