

Biosurfactant production by bacteria isolated from automobile repair shop and its potential applications

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Abstract

Biosurfactants are surface-active compounds produced by microorganisms, primarily during the stationary phase of growth and are necessary for industrial applications, and eco-friendly alternatives to synthetic surfactants. These biodegradable surfactants emulsify hydrocarbons and have diverse applications, including environmental cleanup. This study focuses on microbial-derived biosurfactants from bacteria isolated from soil samples in automobile repair zones. Four bacterial colonies (BS1, BS2, BS3, and BS4) were isolated and screened for biosurfactant production. The selected colony underwent microscopic and biochemical identification. Biosurfactants were produced in a mineral salt medium (MSM) at 37°C for seven days, optimizing incubation time (3, 5, and 7 days), pH (5, 7, and 9), and carbon sources (glycerol, glucose, and petrol). The highest production occurred with petrol at pH 7 after seven days. Fivefold biosurfactant yield of 4 g/L was observed after optimization of biosurfactant production process. Biosurfactants were recovered through acid precipitation and tested for stain removal and oil degradation, demonstrating promising bioremediation applications.

Keywords: Surfactants, Hydrocarbon, Environmental pollution, Mineral salt medium, *Bacillus*

Microorganisms, plants, and animals produce nearly 2000 amphiphilic biological surfactants. These surface-active biomolecules have garnered significant attention due to their properties, such as selectivity, low toxicity, and ease of production, making them valuable in petrochemicals, mining, fertilizers, food, beverages, cosmetics, and pharmaceuticals¹. They serve as emulsifiers, foaming agents, detergents, and functional food ingredients, and low interfacial

surface tension is crucial for oil recovery and bioremediation of crude oil². Biosurfactants also increase the bioaccessibility of hydrophobic substrates through desorption or solubilization and influence bacterial attachment and detachment. Their dual polarity (hydrophilic and hydrophobic regions) enables them to accumulate at fluid-fluid interfaces, such as hydrocarbons and water, thereby reducing interfacial tension³. These advantages over synthetic surfactants, particularly in terms of environmental sustainability and public health, make biosurfactants increasingly desirable for industrial use⁴.

Biosurfactants play a crucial role in pharmaceuticals, oil industry, and environmental remediation. In pharmaceuticals, they are utilized for antimicrobial, antifungal, antiviral, and anticancer properties. Rhamnolipids and other biosurfactants have been demonstrated to prevent the antifungal effects of other substances like iturin⁵. Other biosurfactants, like mannosylerythritol lipids, display antibacterial activity, particularly against Gram-positive bacteria, making them potential therapeutic agents for treating diseases⁶. Their amphiphathic structure is essential for emulsification, foam formation, and oil dispersion, making them significant molecules for food, petroleum, and cosmetics industries⁷. Additionally, biosurfactants produced by marine *Bacillus circulans* have shown strong antimicrobial action against multidrug-resistant strains, proving them as potential candidates⁸. In the oil industry, biosurfactants play a pivotal role in oil extraction, refining, and upgrading due to their capacity to lower interfacial tension. Their amphiphilic nature enhances their effectiveness in emulsifying, dispersing oil, and improving biofouling control⁹. Moreover, biosurfactants incorporated in phytoremediation enhance the metal-tolerant microorganisms to remove heavy metals from the leached sites¹⁰. This aids in environmental cleanup and promotes the sustainable use of biosurfactant-producing organisms for metal detoxification¹¹.

Materials and Methods

Isolation of bacteria¹²

The soil sample was obtained from an automobile repair shop in Woraiyur, Trichy. The sample was

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serially diluted and cultured on nutrient agar medium and subsequently preserved at 4°C for further analysis. Mineral salt medium (MSM) having Na₂HPO₄ (1.73 g/L), KH₂PO₄ (0.68 g/L), NH₄NO₃ (1.0 g/L), NaCl (4.0 g/L), MgSO₄ (0.1 g/L), CaCl₂(0.02 g/L), FeSO₄(0.03 g/L), glucose (5.0 g/L) was employed as the production medium for biosurfactant synthesis. The isolated pure culture was inoculated into MSM and incubated at 37°C for 7 days to facilitate biosurfactant production.

Screening for the presence of bacterial biosurfactants

Drop collapse test¹³

The drop collapse test was a quick and simple method for screening of microbial-biosurfactant production. Biosurfactants diminish surface tension, causing a drop of liquid to spread or collapse on a hydrophobic surface, unlike without them, which retains its shape. In this procedure, microscopic slides were cleaned with ethanol, and labeled as control (C) and test (T). The coconut oil and cell-free supernatant were mixed (1:1) using a glass rod, and the results were observed for drop collapse.

Emulsification test¹⁴

The Emulsification index (E24) was used to assess biosurfactant production by measuring their ability to stabilize emulsions. In this test, a mixture of cell-free supernatant and oil is vortexed to form an emulsion and then left undisturbed for 24 hours. The height of the emulsion layer is measured, and the E24 is calculated using the formula:

$$\text{Emulsification index (E24)} = \frac{\text{Height of emulsion layer}}{\text{Total height of solution}} \times 100$$

A higher E24 value indicates effective biosurfactant activity, with a stable emulsion layer after 24 hours confirming the presence of biosurfactants.

Phosphate test¹⁵

The Phosphate test was used to identify phospholipid-based biosurfactants by detecting the presence of phosphate groups. Ammonium molybdate was added to the sample slightly acidified with concentrated sulfuric acid, forming phosphomolybdate. Hydrazine was then used to reduce the phosphomolybdate in an acidic environment. A yellow precipitate formed due to the reaction of ammonium phosphomolybdate, indicating the presence of

phosphate ions and confirming that the biosurfactant contains phospholipids. A mild application of heat can help the precipitate form more effectively.

Biuret test¹⁶

For lipopeptide-based biosurfactants, 2 mL of cell-free supernatant was heated at 70°C for 10 minutes. After cooling, 10 drops of 1M NaOH solution were added, followed by the dropwise addition of 1% copper sulfate. The appearance of a violet or pink ring at the interface confirms the presence of lipopeptides, resulting from the interaction between copper ions and lipopeptides in an alkaline environment, which forms a coloured complex. This indicates the presence of protein-like biosurfactants.

Characterization of bacteria¹⁸

Microscopic analyses

For gram staining, a heat-fixed smear of the test culture was stained with crystal violet, followed by Gram's iodine, decolorization with ethyl alcohol, and counter-staining with safranin. The bacterial morphology was then viewed using a light microscope. In the Hanging Drop Method, a drop of the test culture was placed on a Vaseline-edged cover slip. A cavity slide was placed over it, inverted, and examined under 40X magnification to visualize bacterial motility.

Biochemical analyses

The Indole test was performed by inoculating tryptone broth and using Kovac's reagent as indicator with red layer indicating a positive result. In the Methyl Red test and Voges-Proskauer test, Glucose phosphate broth was used and methyl red reagent and Barrit's reagent was used as indicator respectively. For the citrate utilization test, blue colouration in simmons citrate agar indicated a positive outcome. The catalase activity was confirmed by bubble formation with hydrogen peroxide addition. The starch hydrolysis test indicates amylase production through zone of clearance. The Triple Sugar Iron test identified preferable sugar uptake and fermentation in the medium which will aid in determining the metabolic profile of the bacterial strains as well as their effectiveness in utilizing various carbon sources, critical for their identification and potential use.

Production and recovery of biosurfactants¹⁷

Sterilized MSM production medium (pH 7.0) was inoculated with biosurfactant-producing bacteria and

incubated at 37°C for 7 days. After incubation, the biosurfactant was recovered using acid precipitation method. The cell-free supernatant (CFS) was separated by centrifugation, then its pH was adjusted to 2.0 and incubated overnight at 4°C. Later, the chloroform-ethanol solvent mixture (2:1 v/v) was added and centrifuged at 5000 rpm for 10 minutes to separate the precipitated biosurfactants. The concentration of biosurfactant produced was measured using the gravimetric method, where the extracted biosurfactant was dried and weighed.

Optimization of biosurfactant production¹⁹

The optimization of fermentation was executed by varying fermentation conditions in a minimal salt medium, including pH levels (5, 7, and 9), incubation times (3, 5, and 7 days), and carbon sources (glycerol, petrol, and glucose) added to the sterilized MSM. Each parameter was adjusted independently while keeping others constant. After incubation at 37°C, the biosurfactant was recovered, and the yield was measured.

Characterization of biosurfactants by GC-MS analysis²⁰

GC-MS analysis was conducted using a SHIMADZU/QP2020GC system, equipped with an AOC-20i autosampler and gas chromatograph interfaced with a mass spectrometer. The study used a 1.2 mL/min injection rate, a 250°C injector temperature, and a 200°C ion source temperature. The oven temperature was set from 50°C (isothermal for 2 minutes) to 280°C. Mass spectra were recorded for ions ranging from 50 to 500 Da, with a scanning interval of 0.3 seconds. The mass spectrum data were analyzed using the WILEY8LIB database to identify the compounds in the biosurfactant sample.

Formulation and applications of biosurfactants

Zinc oxide powder was used as a binder or filler, sodium hydroxide was employed as a builder, and acetic acid was added to modify the pH to create the biosurfactants²¹. These components were mixed with the synthesized biosurfactant to create a formulation suitable for various applications.

Stain removal²²

Castor oil, coffee, and food colourant were applied to fabrics, with surf powder as the control. A mixture of sodium hydroxide, zinc oxide, acetic acid and biosurfactant were added to the powder. The stained

fabrics were soaked for 15 minutes, rinsed in water, and the stain removal was assessed.

Oil degradation²²

Petrol was added to sterilized MSM broth at 2% (v/v), and bacterial growth was monitored over 7 days to assess oil degradation. Optical density (OD) at 600 nm was used to track microbial growth. Residual petrol concentration was determined by centrifuging the broth, recovering petrol from the supernatant via ethanol drying, and calculating degradation with the formula:

$$\begin{aligned} \text{Weight of residual petrol} &= \text{Weight of residue along with the tube} \\ &\quad - \text{Weight of empty tube} \\ \text{Amount of oil degraded} &= \text{Amount of petrol added} \\ &\quad - \text{Amount of residual petrol} \end{aligned}$$

$$\% \text{ Oil Degradation} = \frac{\text{Amount of petrol added} - \text{Amount of residual petrol}}{\text{Amount of petrol added}} \times 100$$

Results and Discussion

Isolation of microorganisms

The soil sample (Fig. 1A) from the engine oil spilled zones of automobile repair zones was selected for sampling. Low moisture content, significant hydrocarbon contamination, and enhanced heavy metal concentrations (such as lead, cadmium, and zinc) as a result of long-term exposure to petroleum products are typical physicochemical characteristics of soil from an automobile shed. Because of oil spills, the pH is frequently neutral to slightly alkaline, and there is an abundance of organic carbon. Although the soil's microbial diversity is minimal, it might include bacteria that break down hydrocarbons. The colony forming units obtained after serial dilution was enumerated and found to be 25 CFU/mL²³ (Fig. 2). The colonies were mucoid and white coloured. The pure cultures of four different strains (BS1, BS2, BS3



Fig. 1 — Oil spilled soils; colonies after serial dilution of the soil sample.

& BS4) were obtained and it was used for further analysis.

Screening of biosurfactant producing microorganisms

The screening tests revealed the BS4 strain showed positive for drop collapse test with maximum emulsification index value (Fig. 3) among the four strains. Fig. 4 reveals the results of biuret and phosphate tests for all BS strains. It was seen that all strains have biosurfactant producing potential notably lipopeptides. Among all, BS4 strain was selected for further analysis due to higher emulsification index.

Characterization of microorganisms

Based on the microscopic and biochemical analysis, the potential biosurfactant producer was found to be *Bacillus* species. Fig. 5 shows the microscopic image of BS4 Gram positive, non-motile strain under 40X magnification which inturn supports the identification of the biosurfactant producer as *Bacillus* species. The

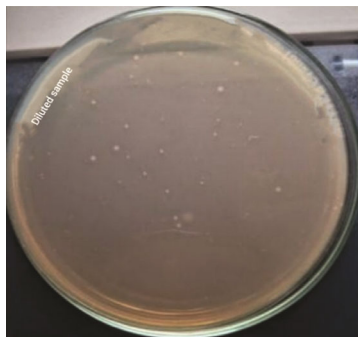


Fig. 2 — Oil spilled soils; colonies after serial diultion of the soil sample.

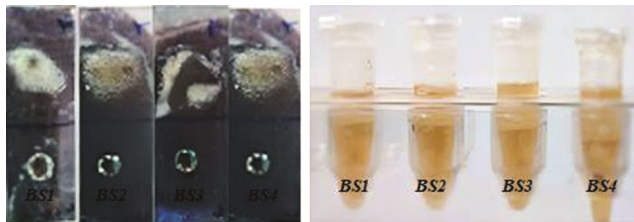


Fig. 3 — (A & B) Screening of Biosurfactant producing Organisms



Fig. 4 — (A & B) Type of biosurfactants produced by bacteria

biochemical results shown in Table 1 indicates the characteristic features of *Bacillus* genus which was obtained by the standard protocols from Bergey’s manual of systematic bacteriology²⁴.

Production and optimization of biosurfactant production

The biosurfactant yield of BS4 was found to be 3.1 g/L followed by optimization of pH, incubation time and carbon source of the medium. Fig. 6 shows the biosurfactant yield after optimization. The maximum biosurfactant yield was obtained under neutral pH; 3 days of incubation and petrol or glucose supplemented MSM. The experiments were conducted using glycerol, petrol, and glucose as carbon sources at pH 5, 7, and 9, and at temperatures of 30°C, 37°C, and 45°C. The highest biomass production (3.8 g/L) was observed with glucose at pH 7 and 37°C, whereas the maximum biosurfactant yield (2.5 g/L) was obtained with petrol at pH 7 and 37°C. The biosurfactant yield was enhanced from 0.8 g/L to 4.0 g/L (5 folds increase) after production process was carried out with optimal process conditions. Biomass and biosurfactant production were significantly lower

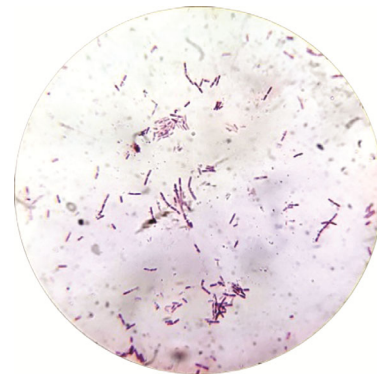


Fig. 5 — Gram staining image of BS4 strain.

Table 1 — Biochemical characteristics of BS4 strain		
Biochemical tests	Observation	Inference
Gram’s staining	Rod shaped purple bacterium	Gram positive
Indole test	No red ring formation	Negative
Methyl red test	No red colour formation	Negative
Vogus-Proskauer test	No pink colour formation	Negative
Citrate utilization test	Blue to green	Positive
Starch hydrolysis test	Zone of clearance	Positive
Catalase test	Bubble formation	Positive
TSI test	Glucose fermentation without gas formation	K/A
Motility test	No movement	Non-motile

at extreme pH and temperature conditions. To improve stability and efficiency, the biosurfactant formulation was standardized using emulsification traits, stabilizers, and pH modifications. The efficacy for industrial and environmental applications was established by purifying it using solvent extraction and vacuum drying, and testing its stability across changes in temperature, pH, and ionic strength.

Characterization of biosurfactants

The produced biosurfactant was characterized by Gas chromatography Mass Spectrometry analysis.(Fig. 7). The GC-MS spectrum revealed 25 different compounds based on the mass to charge ratio. Among the bio-actives, cyclopentanecarboxylic acid, 1-adamantylmethyl ester, tridecanoic acid, methyl ester, methyl 6-hydroxycaproate, and 13-docosenamide, (z)- were likely to act as biosurfactants. The compound,13-docosenamide, (z)- was found to be the most prominent lipopeptide biosurfactant with a concentration of 75.03%²⁵ having

potential to reduce surface tension due to functional groups such as fatty acid esters and fatty acid amides²⁶.

Application of biosurfactants

The stain-removing potential of the synthesized biosurfactants was assessed and it was shown in Fig. 8. The BS4 strain inoculated in MSM broth supplemented with 2% petrol as a carbon source and it was incubated over 3 weeks at 37°C. The optical

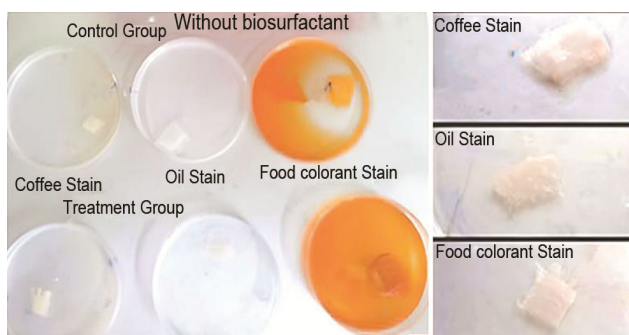


Fig. 8 — Stain removal application.

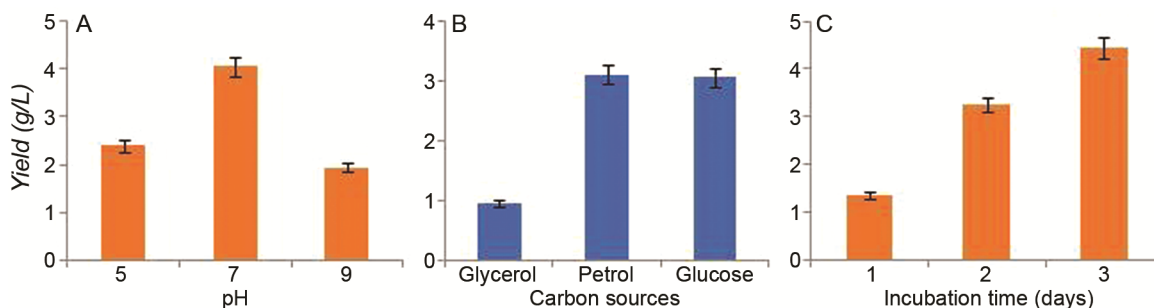


Fig. 6 — Optimization of Biosurfactant production by BS4 strain. Optimization of pH (A); Optimization of carbon sources (B); Optimization of incubation time (C).

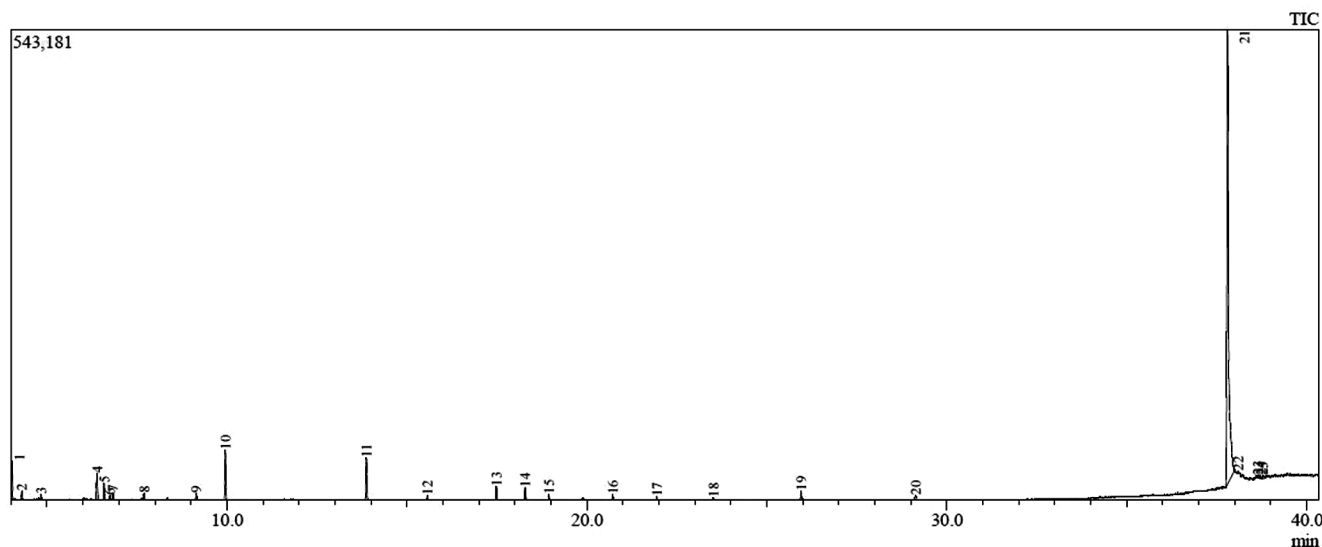


Fig. 7 — GCMS spectrum of produced biosurfactant.

density at 600 nm was measured at every 7 days interval. The growth curve obtained from this approach was shown in Fig. 9.

The results confirmed that the bacteria utilizes petrol (hydrocarbon) as a nutrient source for enhancing its growth rate. The bacterial concentration increases as the time proceeds, which indicates the bacterial strain utilizes the petrol source to synthesize energy for its metabolism and growth. The residual oil concentration was found to be 89.8%. Higher oil degradation percentage confirms the utilization of petrol hydrocarbon source.

Microorganisms isolated from oil-contaminated soils near automobile repair zones demonstrated significant biosurfactant production potential. Using serial dilution techniques, 25 CFU/mL were observed, reflecting microbial adaptation to hydrocarbon-rich environments. The isolation of diverse colony types, including white and mucoidal forms, suggests the presence of bacteria capable of metabolizing hydrocarbons. Recent studies support the efficacy of serial dilution in microbial isolation from polluted environments, emphasizing its role in assessing bioremediation potential²⁷. The adaptability of microbes in such conditions is also highlighted in recent research, which shows their ability to survive and thrive in the presence of hydrocarbons²⁸.

Strain BS4 showed the highest potential for biosurfactant production, based on positive results from drop collapse and emulsification index assays. These assays are widely accepted for screening biosurfactant producers, particularly in hydrocarbon-contaminated environments, where bacteria are often capable of reducing surface tension²⁹. BS4 was identified as a *Bacillus* species, a genus well-known for producing lipopeptides like surfactin, which is efficient in stabilizing emulsions and breaking down hydrocarbons. Recent findings confirm that *Bacillus*

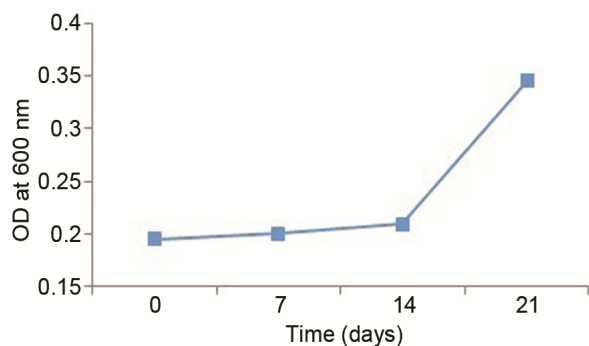


Fig. 9 — Growth curve of BS4 strain.

strains from polluted sites possess unique metabolic traits that enhance biosurfactant production³⁰.

The production of biosurfactants by BS4 yielded 3.1 g/L, which increased under optimized conditions (pH 7, 7 days incubation, with petrol or glucose as carbon sources). This aligns with recent research showing that neutral pH and hydrocarbons like petrol can enhance biosurfactant production³¹. Characterization of synthesized biosurfactants by GC-MS analysis identified 25 compounds in the biosurfactant, with 13-docosenamide (z)- being the most prominent at 75.03%. Studies suggest that fatty acid esters and amides, such as 13-docosenamide (lipopeptide), have strong surface tension-reducing properties, making them highly effective in applications like oil degradation³².

In practical applications, the biosurfactants produced by BS4 were highly effective in stain removal and oil degradation studies, achieving an 89.8% reduction in oil concentration. This finding is consistent with other research showing high efficiency in oil degradation by biosurfactant-producing bacteria, particularly in the context of environmental remediation³³. The increasing focus on sustainable, eco-friendly alternatives to chemical surfactants further highlights the importance of microbial biosurfactants in bioremediation and other industrial applications³⁴.

Conclusion

The isolation of *Bacillus* strain BS4 from oil-contaminated soils demonstrated its high biosurfactant production potential, with significant emulsification and hydrocarbon degradation capabilities, positioning it as a candidate for environmental applications like bioremediation. Optimization of production conditions, including pH and carbon source, increased the biosurfactant yield, whereas GC-MS analysis identified lipopeptides with strong surface tension-reducing properties. Future research should focus on advanced bioprocess engineering, genetic modification of biosurfactant-producing strains, and exploring new microbial sources to improve efficiency. Collaborative efforts between regulatory bodies, industry, and academia are crucial for scale up and facilitating commercial applications, driving sustainable environmental solutions.

The potent BS4 strain could be genetically engineered to produce biosurfactant for scale-up and

utilization of biosurfactant in industries. Future studies should concentrate on co-culturing techniques for better biodegradation, improving fermentation conditions using statistical models, and genetically modifying the isolated strains to increase biosurfactant synthesis. Key biosynthetic genes for surfactant synthesis might be identified by whole-genome research, and industrial applications could be facilitated by large-scale production studies.

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Conflict of interest

The authors have no conflict of interest to declare.

References

- Adetunji AI & Olaniran AO. Production and potential biotechnological applications of microbial surfactants: An overview. *Saudi J. Biol. Sci.*, 28 (2021) 669.
- Sarubbo LA, Maria da Gloria CS, Durval, IJB, Bezerra KGO, Ribeiro BG, Silva IA & Banat I. Biosurfactants, Production, properties, applications, trends, and general perspectives. *Biochem. Eng. J.*, 181 (2022) 108377.
- Kwon JH, Ji MK, Kumar R, Islam MM, Khan MA, Park YK & Jeon BH. Recent advancement in enhanced soil flushing for remediation of petroleum hydrocarbon-contaminated soil: a state-of-the-art review. *Rev Environ Sci Biotechnol*, 22 (2023) 679.
- Kumari V. Biosurfactant as an antimicrobial and biodegradable agent a review. *Microbial Surfactants*, (2022) 19.
- Thakur S, Singh A, Sharma R, Aurora R & Jain SK. Biosurfactants as a novel additive in pharmaceutical formulations: current trends and future implications. *Curr. Drug Metab*, 21 (2020) 885.
- Coelho ALS, Feuser PE, Carciofi BAM, de Andrade CJ & de Oliveira D. Mannosylerythritol lipids: antimicrobial and biomedical properties. *Appl Microbiol. Biotechnol*, 104 (2020) 2297.
- Davies A & Amin S. Rheology of Cosmetic Products: Surfactant Mesophases, Foams and Emulsions. *J. Cosmet. Sci.*, 71 (2020).
- Sajayan A, Ravindran A, Selvin J, Ragothaman P & Seghal Kiran G. An antimicrobial metabolite n-hexadecenoic acid from marine sponge-associated bacteria *Bacillus subtilis* effectively inhibited biofilm forming multidrug-resistant *P. aeruginosa*. *Biofouling*, 39 (2023) 502.
- Chafale A & Kapley A. Biosurfactants as microbial bioactive compounds in microbial enhanced oil recovery. *J. Biotech*, 352 (2022) 1.
- Alves AR, YinQ, Oliveira RS, Silva EF & Novo LA. Plant growth-promoting bacteria in phytoremediation of metal-polluted soils: current knowledge and future directions. *Sci. Total Environ*, 838 (2022) 156435.
- Kashif A, Rehman R, Fuwad A, Shahid MK, Dayarathne HNP, Jamal A & Choi Y. Current advances in the classification, production, properties and applications of microbial biosurfactants-A critical review. *Adv. Colloid Interface Sci.*, 306 (2022) 102718.
- Nayarisseri A, Khandelwal R & Singh SK. Screening and characterization of Lipopeptide Biosurfactant Producing *PaenibacillusDendritiformis* and its applicability for enhanced oil recovery.(2021). Research gate. DOI: 10.21203/rs.3.rs-871557/v2
- Mishra N, Agsar D, Deepthi SS, Kattagouda UM, Shetty PR & Pandey V. Novel application of *Nerium* leaf and Image J software in drop collapse assay for rapid screening of biosurfactant producing microorganisms. *Indian J. Exp. Biol.*, 59 (2021) 484.
- Ribeiro BG, dos Santosb MM, da Silvaca IA, Meirad HM, de Oliveirad AM, Guerrab JM & Sarubbod LA. Study of the biosurfactant production by *Saccharomyces cerevisiae* URM 6670 using agroindustrial waste, *Chem Eng*, 79 (2020) 61.
- Patel K & Patel FR. Screening of biosurfactant producing yeasts isolated from mangrove ecosystem of Surat region of Gujarat, India. *Indian J. Sci. Technol*, 13 (2020) 204.
- Bellebcir A, Merouane F, Chekroud K, Bounabi H, Vasseghian Y, Kamyab H & Berkani, Bioprospecting of biosurfactant-producing bacteria for hydrocarbon bioremediation: Optimization and characterization. *Korean J. Chem. Eng.*, 40 (2023) 2497.
- Aboelkhair H, Diaz P & Attia A. Biosurfactant production using Egyptian oil fields indigenous bacteria for microbial enhanced oil recovery. *J petrol sci eng*, 208 (2022) 109601.
- Kathiravan N, Rajesh A, Kim JW & Davoodbasha M. Isolation and Characterization of Biosurfactant-Producing Soil Fungus *Penicillium sp.* *Appl Biochem Biotechnol*, 196 (2024) 3234.
- Sun W, Zhu B, Yang F, Dai M, Sehar S, Peng C & Naz I. Optimization of biosurfactant production from *Pseudomonas sp.* CQ2 and its application for remediation of heavy metal contaminated soil. *Chemosphere*, 265 (2021) 129090.
- Ghazi Faisal Z, Sallal Mahdi M & Alobaidi KH. Optimization and chemical characterization of biosurfactant produced from a novel *Pseudomonas guguensis* strain Iraqi ZG. *International J. Microbiol.*, 1 (2023) 1571991.
- Drakontis CE & Amin S. Biosurfactants, Formulations, properties, and applications. *Curr. Opin. Colloid Interface Sci.*, 48 (2020) 77.
- Anidu FN, Uba BO, Ezemba CC, Okoye EL & Dokubo CU. Study on Optimization, Degreasing and Destaining Potentials of Glycophospholipid Biosurfactant Produced by *Bacillus*. *DUJOPAS*, 9 (2023) 29.
- Levicana A & Hinton Jr A. CAMPYAIR, a new selective, differential medium for *Campylobacter spp.* isolation without the need for microaerobic atmosphere. *Microorganisms*, 10 (2022) 1403.
- Singh J & Singh SP. Isolation and identification of *bacillus* species from soil for phosphate, potassium solubilisation and amylase production. *Int. J. Curr. Microbiol. Appl. Sci.*, 9 (2020) 415.
- Alvionita DN, Rahayu SRI & Mubarak NR. Characterization, identification, and analysis of bioactive compound of endophytic bacteria from *Hoya multiflora Blume*. *Biodiversitas*, 21 (2020) 195.

- 26 Nagtode VS, Cardoza C, Yasin HKA, Mali SN, Tambe SM, Roy P & Pratap AP. Green surfactants (biosurfactants): a petroleum-free substitute for Sustainability Comparison, applications, market, and future prospects. *ACS omega*, 8 (2023) 11674.
- 27 Ansari AT. Biosorption and Discolorization of Textile Dye Effluent Using Fungi Isolated From Soil Samples Collected Near Textile Dye Industry. *Microbial Biofilms*, (2020) 24 .
- 28 Kebede G, Tafese T, Abda EM, Kamaraj M & Assefa F. Factors influencing the bacterial bioremediation of hydrocarbon contaminants in the soil mechanisms and impacts. *J. Chem*, 1 (2021)9823362.
- 29 Gurkok S & Ozdal M. Screening Methods for Biosurfactant-Producing Microorganisms. *Microbial Biosurfactants*, (2023) 1.
- 30 Ray S, Sankhyan S, Sonkar M & Kumar P. Role of biosurfactants in the remediation of emerging pollutants. In *Management and Mitigation of Emerging Pollutants*, (2023) 411.
- 31 Yin J, Wei X, Hu F, Cheng C, Zhuang X, Song M & Ma A. Halotolerant *Bacillus velezensis* sustainably enhanced oil recovery of low permeability oil reservoirs by producing biosurfactant and modulating the oil microbiome. *Chem. Eng. J*, 453 (2023) 139912.
- 32 Hernández-Fernández J, Puello-Polo E & López-Martínez J. Recovery of (Z)-13-docosenamide from industrial wastewater and its application in the production of virgin polypropylene to improve the coefficient of friction in film type applications. *Int. J. Sustain*, 15 (2023) 1247.
- 33 Ibrahim S, Abdul Khalil K, Zahri KNM, Gomez-Fuentes C, Convey P, Zulkharnain A & Ahmad SA. Biosurfactant production and growth kinetics studies of the waste canola oil-degrading bacterium *Rhodococcuserythropolis* AQ5-07 from Antarctica. *Molecules*, 25 (2020) 3878.
- 34 Eras-Muñoz E, Farré A, Sánchez A, Font X & GeaT. Microbial biosurfactants: a review of recent environmental applications. *Bioengineered*, 13 (2022)12365.