



Extraction and characterization of Biosurfactant produced by *Kocuria marina*

Aditi Gamit¹, Rashmi Bariya² and Mehul Dave³

¹PG student, Department of life science, Indian Institute of Teacher Education (IITE), State public university, Gandhinagar-382016, Gujarat, India

²Assistant Professor, Department of life science, Centre of Education, Indian Institute of Teacher Education (IITE), State public University, Gandhinagar-382016, Gujarat, India

³Associate Professor, Department of life science, Centre of Education, Indian Institute of Teacher Education (IITE), State public University, Gandhinagar-382016, Gujarat, India

Abstract

The increasing demand for eco-friendly and biodegradable surfactants has driven the search for efficient microbial biosurfactants as sustainable alternatives to synthetic chemicals. The present study reports the isolation, screening, optimization and molecular characterization of a biosurfactant-producing bacterium recovered from petroleum-contaminated soil. Bacterial isolates were obtained via serial dilution and spread-plate techniques on agar media, with pure cultures established through the streak-plate method and subsequently activated in nutrient broth. Primary and secondary screening confirmed biosurfactant-producing activity in the active culture. Optimization of biosurfactant yield was carried out by evaluating the influence of varying pH conditions, nitrogen sources, and carbon sources on production efficiency. The biosurfactant was extracted using the acid precipitation method. The biosurfactant producing organism was identified as *Kocuria marina* using MALDI-TOF MS. The extracted Biosurfactant exhibited characteristics consist with a glycolipid-type biosurfactant, possibly rhamnolipid like in nature. These results establish *Kocuria marina* as a promising candidate for Glycolipid type biosurfactant production with potential applications in bioremediation and industrial biotechnology. Future studies should focus on process scale-up and evaluation of its performance under field and industrial conditions.

Keywords: Biosurfactant, Petroleum-contaminated soil, *Kocuria marina*, Rhamnolipid, MALDI-TOF, HPLC, GC-MS.

Introduction

Biosurfactants are amphiphilic microbial compounds containing both hydrophilic and hydrophobic groups that reduce surface and interfacial tension between different phases (Sarubbo *et al.*, 2022). These biomolecules possess important properties such as emulsification, detergency, foaming, and oil dispersion, which make them useful in environmental, pharmaceutical, petroleum, cosmetic, and food industries (Fakruddin, 2012). Unlike synthetic surfactants, biosurfactants are biodegradable, less toxic, environmentally friendly, and stable under extreme conditions such as high salinity, temperature, and pH (Makkar *et al.*, 2011).

Marine microorganisms are considered important sources of novel biosurfactants because they survive in saline and stressful environments, which enhances the production of unique bioactive metabolites (Sarubbo *et al.*, 2022). *Kocuria marina* is a Gram-positive, aerobic, halotolerant marine actinobacterium isolated from marine sediment and capable of tolerating high salt concentrations (Kim *et al.*, 2004). Due to its adaptation to marine conditions and unique cellular characteristics, *Kocuria marina* may have potential for biosurfactant production and industrial applications.

Although biosurfactant production has been widely studied in genera such as *Pseudomonas*, *Bacillus*, and *Candida*, limited studies are available on biosurfactant extraction and characterization from *Kocuria marina*. The extracted biosurfactant from *Kocuria marina* can be further explored for large-scale production and its applications in bioremediation, oil recovery, pharmaceuticals, agriculture, cosmetics, and other environmentally sustainable industrial processes.

Materials and Methods

An Oil-contaminated soil sample was collected from Road Number 6, Sugandhi, Sector 23, Gandhinagar, Gujarat-382016. The sample was

serially diluted and inoculated on Nutrient Agar (N agar) medium followed by incubation at 37°C for 48 h for bacterial isolation (Jaysree *et al.*, 2011).

Distinct colonies were selected based on morphological characteristics and purified by repeated streaking on fresh N agar plates to obtain pure cultures (Nayarisseri *et al.*, 2018).

Nutrient broth medium was prepared using 125 mL distilled water, 0.625 gm peptone, 0.625 gm sodium chloride, 0.25 gm yeast extract and 0.125 gm meat extract, followed by sterilization through autoclaving. A selected bacterial colony from the pure culture was inoculated into the nutrient broth medium and incubated at 37°C for 48 h to obtain an active bacterial culture (Jaysree *et al.*, 2011).

Primary screening was carried out using drop collapse assay and oil displacement test. In the drop collapse assay, 1 mL active culture was centrifuged at 2000 rpm for 0 min, and the supernatant was added onto crude oil on a glass slide to observe drop collapse activity. In the oil displacement test, 20-25 mL distilled water and crude oil were added to a petri plate, followed by 30-50 µL culture supernatant. Formation of a clear zone after 1-2 min indicated biosurfactant production (Chithra & Hemashenpagam, 2021).

Secondary screening was performed using phenol sulphuric acid test and emulsification assay. In the phenol sulphuric acid test, 2 mL supernatant was mixed with 5% phenol followed by dropwise addition of sulphuric acid and colour change was observed. For emulsification assay, 2 mL crude oil was added to 2 mL culture supernatant, vortexed for 2 min, and kept at room temperature to measure the height of the emulsion layer (Saikia, 2012).

Optimization of biosurfactant production was carried out using nutrient broth medium adjusted to different pH values (4.5, 7 and 8.5) using HCl and NaOH after sterilization. Selected bacterial colonies were inoculated into the prepared media and incubated on a shaker at 150 rpm at room temperature for 48 h (Saikia, 2012). Biosurfactant

production at different pH conditions was evaluated by secondary screening methods.

Optimization of biosurfactant production was carried out using two different media compositions prepared in 100 mL distilled water containing nutrient broth supplemented with 0.5 gm NaNO₃ and 2 gm glucose. After sterilization, selected bacterial colonies were inoculated into the media and incubated at 37°C for 48 h. The cultures were centrifuged at 6000 rpm for 2 min (Sarubboet *et al.*, 2022), and the obtained supernatant was used to evaluate biosurfactant production through secondary screening methods.

The biosurfactant-producing bacterium was identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry at the Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, Gujarat, India. Pure bacterial culture was obtained by repeated streaking on nutrient agar plates followed by incubation at 37°C for 24 h. A single isolated colony was subjected to MALDI-TOF MS analysis, and the bacterium was identified based on the best match score confirming its taxonomic identity.

Biosurfactant extraction was performed by acid precipitation method. Active culture was centrifuged at 8000 rpm for 10 min, and the collected supernatant was adjusted to pH 2 using HCl and stored at 4°C. Formation of white precipitates confirmed biosurfactant production (Saikia, 2012).

High-Performance Liquid Chromatography (HPLC) analysis of the extracted biosurfactant was carried out at the Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, Gujarat, India. The analysis was performed at 280 nm using acetonitrile and water as the mobile phase

with a flow rate of 0.5 mL/min and a run time of 30 min for characterization of biosurfactant components (Wang *et al.*, 2024).

GC-MS analysis was performed at Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, Gujarat, to identify the components of the extracted biosurfactant. Partially purified biosurfactant (0.1 g) was dissolved in methanol (100 µg/mL) and analysed using a ZB-5MS capillary column with helium as carrier gas at a flow rate of 2 mL/min. The injector and detector temperatures were maintained at 230°C and 280°C, respectively. The column temperature was programmed from 80°C to 280°C at a rate of 8°C/min (Elazzazyet *et al.*, 2015).

Results and Discussion

According to Jaysree *et al.* (2011), bacterial isolates obtained from oil-contaminated soil can be effectively purified and maintained for further screening of biosurfactant-producing activity.

Primary screening for biosurfactant production was performed using drop collapse and oil displacement assays. Out of six isolates, five isolates showed positive results in the drop collapse assay [Table 1], while isolate 11 10⁻⁵ showed no activity.

In the oil displacement assay [Table 2], isolate 1 10⁻¹ exhibited the highest activity (+++), whereas isolates 14 10⁻⁹, 3 10⁻², and 4 10⁻² showed moderate activity (++). Isolate 2 10⁻⁹ showed weak activity (+), confirming the presence of biosurfactant-producing bacterial isolates. Similar screening methods for identification of potential biosurfactant-producing bacteria from oil-contaminated soil were also reported by Saravanan and Vijayakumar (2012).

Table 1: Drop collapse assay

Slide no.	Isolate	Initial shape of oil drop	Observation after 1 or 2 min	Result (+/-)
1.	14×10^{-9}	Beaded	Collapse	+
2.	1×10^{-1}	Beaded	Collapse	+
3.	2×10^{-9}	Beaded	Collapse	+
4.	11×10^{-5}	Beaded	Beaded	-
5.	3×10^{-2}	Beaded	Collapse	+
6.	4×10^{-2}	Beaded	Collapse	+

Figure-1: Drop collapse assay

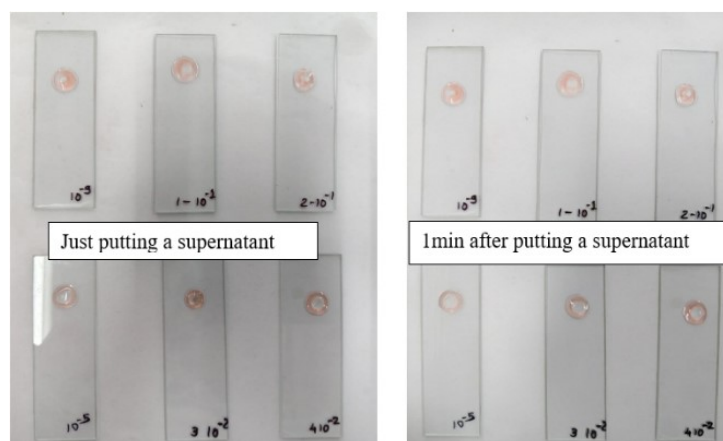
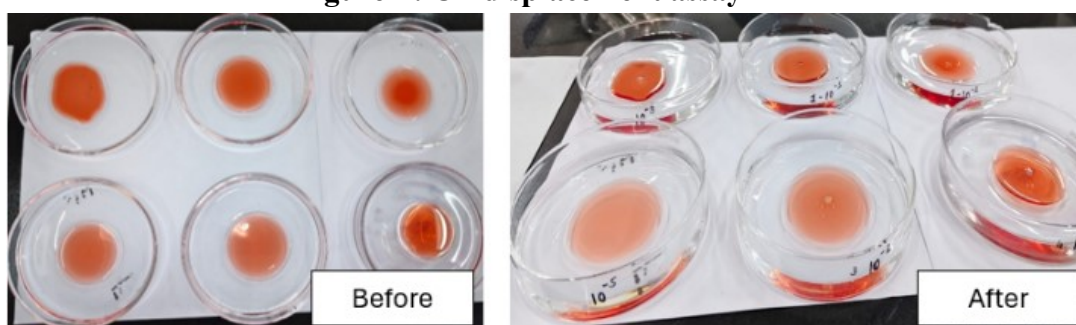


Table 2: Oil displacement assay

Plate no.	Isolate	Volume of CFS in μ l	Visual observations	Oil displacement activity	Result (+/-)
1.	14×10^{-9}	20	Prominent clear zone	Moderate	++
2.	1×10^{-1}	20	Very prominent clear zone	Strong	+++
3.	2×10^{-9}	20	No visible clear zone	Weak	+
4.	11×10^{-5}	20	No clear zone	Negative	-
5.	3×10^{-2}	20	Prominent clear zone	Moderate	++
6.	4×10^{-2}	20	Prominent clear zone	Moderate	++

Figure-2: Oil displacement assay



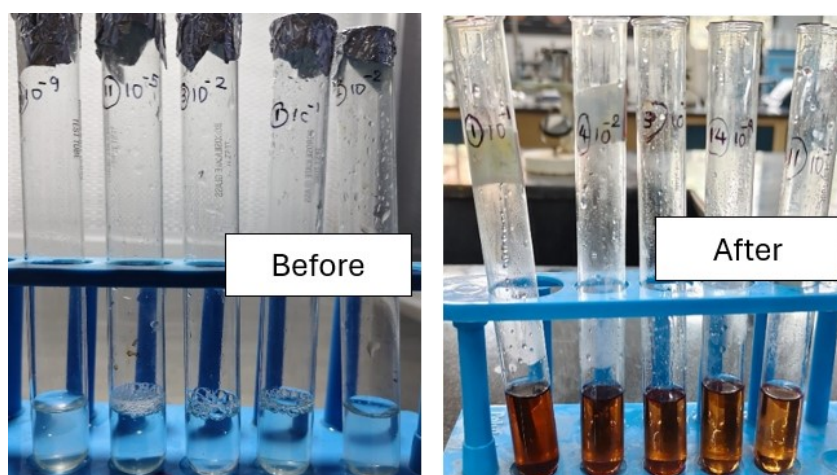
Secondary screening was performed using 2 assays: Phenol acid assay and Emulsification assay. The phenol sulphuric acid assay showed variation in biosurfactant production among the isolates based on OD values at 480 nm. [Table 3] Isolate 10^{-1} exhibited the highest activity (OD

1.92), followed by isolate 10^{-2} (1) (OD 1.37), while the remaining isolates showed lower OD values. The higher absorbance observed in isolate 10^{-1} indicates greater glycolipid biosurfactant production.

Table 3: Phenol acid assay

Isolate	OD at 480 nm	Phenol Sulphuric Acid Assay Result
10-1	1.92	Highest activity
10-2 (1)	1.37	Moderate activity
10-2 (2)	1.00	Low activity
10-9	1.03	Low activity
10-5	0.95	Lowest activity

Figure-3: Phenol acid assay



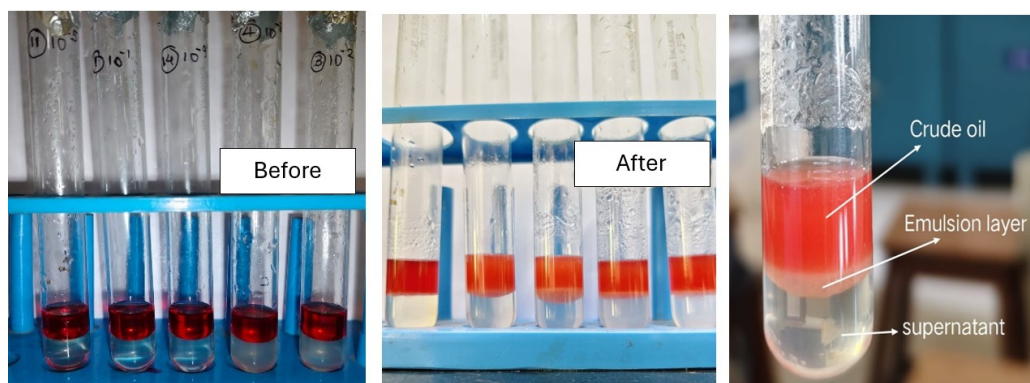
The emulsification index (E₂₄) assay showed variation in emulsifying activity among the bacterial isolates. [Table 4] Isolate 10^{-2} (2) exhibited the highest emulsification index of 30%, followed by isolate 10^{-2} with 25%. Isolate 10^{-9} showed moderate activity with 20%, while

isolates 10^{-1} and 10^{-5} showed lower emulsification activity of 15%. Higher E₂₄ values indicate better emulsification efficiency and biosurfactant production potential (Saravanan & Vijayakumar, 2012; Bodour *et al.*, 2004).

Table 4: Emulsification assay

Isolate	E ₂₄ (%) after 30 min	Activity Level
10^{-1}	15	Low
10^{-2}	25	Moderate
10^{-2} (2)	30	Highest
10^{-9}	20	Moderate
10^{-5}	15	Low

$$E_{24}(\%) = \left(\frac{\text{Height of the emulsified layer}}{\text{Total height of the liquid layer}} \right) \times 100$$

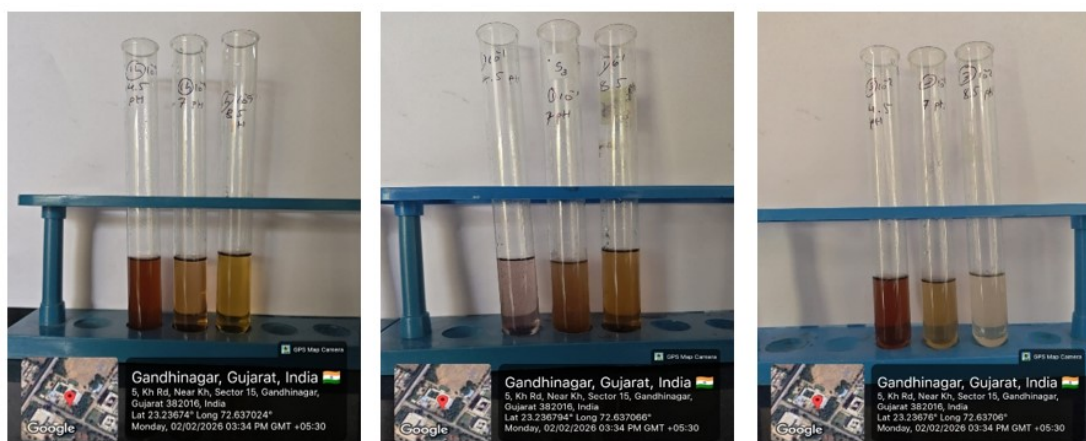
Figure-4: Emulsification assay

The phenol sulphuric acid assay showed variation in biosurfactant production at different pH conditions. [Table 5] Isolate 14 (10^{-9}) exhibited the highest OD value at pH 4.5 (2.75), while isolate 1 (10^{-1}) showed maximum activity at pH 7 (2.09). Isolate 3 (10^{-2}) showed comparatively

higher activity at pH 4.5 (2.10) and gradually decreased at higher pH values. Overall, neutral pH (7) and acidic pH (4.5) supported greater biosurfactant production, whereas alkaline pH (8.5) resulted in reduced activity (Saikia, 2012).

Table 5: Phenol acid assay for optimization of Biosurfactant at different pH

pH	OD at 480 nm of Isolate 14 10^{-9}	OD at 480 nm of Isolate 1 10^{-1}	OD at 480 nm of Isolate 3 10^{-2}
4.5	2.75	0.57	2.10
7.0	0.56	2.09	0.85
8.5	0.50	1.41	0.50

Figure-5: Phenol acid test for different pH optimization

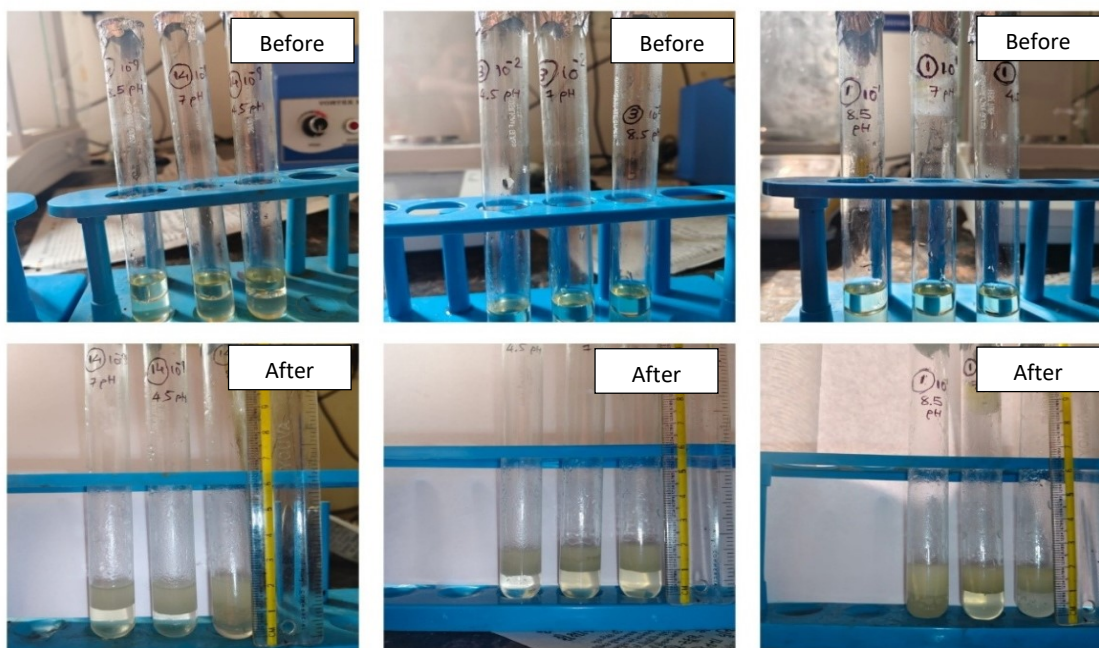
The emulsification index (E24%) varied among the isolates at different pH conditions. Isolate 10^{-9} showed the highest emulsification activity at pH 8.5 with an E24 value of 65%, while isolate 10^{-1} exhibited 60% activity at the same pH. Isolate 10^{-2}

showed stable activity at pH 4.5 and 7.0 (45%) and increased to 55% at pH 8.5. The results indicate that alkaline pH (8.5) favoured maximum biosurfactant production among the tested isolates (Saikia, 2012).

Table 6: Emulsification assay for optimization of Biosurfactant at different pH

pH	E24 (%) of Isolate 10^{-9}	E24 (%) of Isolate 10^{-1}	E24 (%) of Isolate 10^{-2}
4.5	45	40	45
7.0	45	50	45
8.5	65	60	55

Figure-6: Emulsification assay for optimization of Biosurfactant at different pH



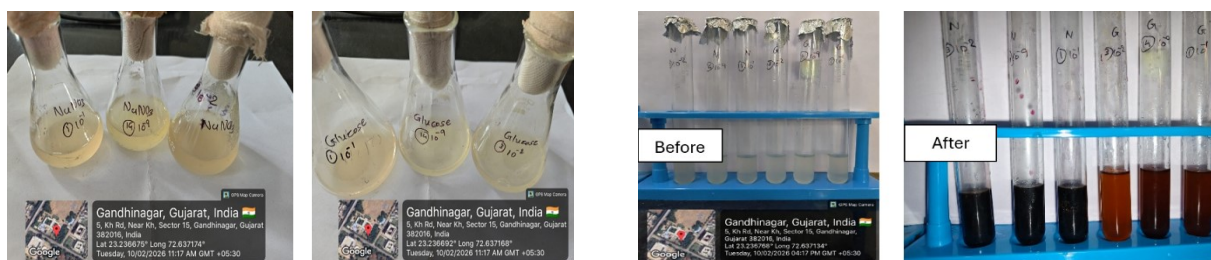
The phenol sulphuric acid assay showed variation in biosurfactant production using different carbon and nitrogen sources. [Table 7] Glucose exhibited consistently high OD values (3.0) for all isolates, indicating enhanced biosurfactant production. In contrast, sodium nitrate showed variable activity,

with isolates 10^{-9} and 10^{-2} exhibiting higher OD values (3.0), while isolate 10^{-1} showed lower activity (1.3). The results suggest that glucose was a more effective source for biosurfactant production compared to sodium nitrate (Sarubbo *et al.*, 2022).

Table 7: Phenol acid test for optimization of Biosurfactant using nitrogen and carbon source

Isolate	OD with Sodium Nitrate	OD with Glucose
10^{-9}	3.0	3.0
10^{-1}	1.3	3.0
10^{-2}	3.0	3.0

Figure-7: Phenol acid assay for optimization of BS using N and C source



Bruker MALDI Biotyper Identification Results



Run Info:

Run Identifier: 260205-1547-211
Comment:
Operator: Admin@FLEX-PC
Run Creation Date/Time: 2026-02-05T15:57:47.828
Number of Tests: 3
Type: Standard
BTS-QC: not present
BTS-QC Position:
Instrument ID: 1857371.00994
Server Version: 4.1.100 (PYTH) 174 2019-06-158_01-16-09

Result Overview

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
J22 (+) (B)	1 (Standard)	Shewanella xiamenensis	1.71	Shewanella xiamenensis	1.70
J23 (-) (C)	3 (Standard)	No Organism Identification Possible	1.33	No Organism Identification Possible	1.33
J24 (+) (B)	14 (Standard)	Kocuria marina	1.78	Kocuria marina	1.74

Biosurfactant extraction by acid precipitation method resulted in the formation of visible white precipitates after centrifugation and acidification of the culture supernatant to pH 2 followed by storage at 4°C. The obtained precipitate

confirmed successful biosurfactant production by the bacterial isolate. Similar observations of white biosurfactant precipitates after acid precipitation were reported by Zhou *et al.* (2019).

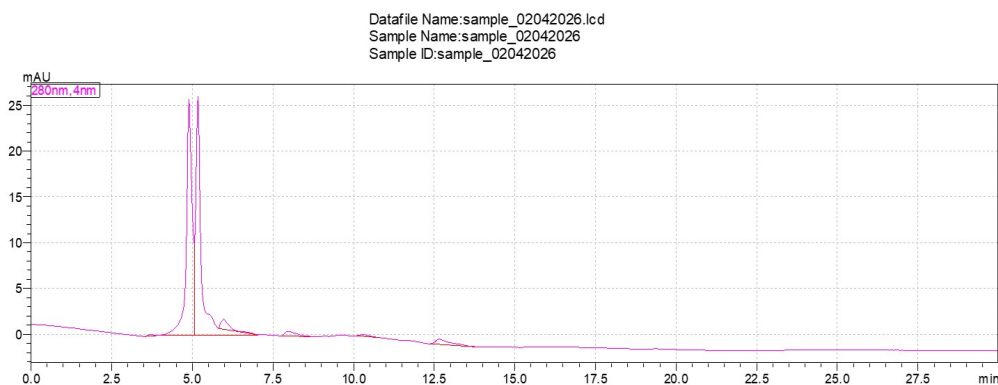
Figure-8: Biosurfactant production



MALDI-TOF MS analysis was carried out for rapid identification of bacterial isolates based on protein mass spectral fingerprints. Among the analyzed samples, 1×10^{-1} was identified as *Shewanella xiamenensis* with score values of 1.71 and 1.70, indicating probable genus-level identification. Sample 5×10^{-3} showed low score values (1.37 and 1.35), and no reliable identification was obtained. Sample 14×10^{-9} was identified as *Kocuria marina* with score values of 1.78 and 1.74, indicating reliable genus-level identification as *Kocuria* Species.

The HPLC chromatogram showed a major peak at approximately 5.0 min, indicating the presence of a dominant biosurfactant compound, likely a glycolipid-type rhamnolipid. Minor peaks observed between 5.5 and 6.5 min may represent different **phenolic compounds** and biosurfactants with aromatic groups. No significant peaks were detected between 8 and 15 min, suggesting the absence or negligible presence of lipopeptide biosurfactants such as surfactin, iturin, and fengycin (Wang et al., 2024).

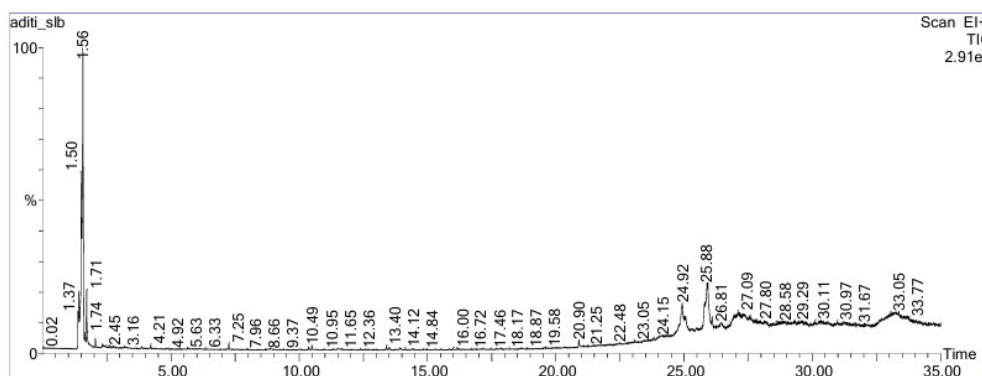
Figure-9: HPLC chromatograms



GC-MS analysis revealed the presence of fatty acid derivatives, hydrocarbons, ester compounds, and trimethylsilyl (TMS) derivatives, indicating lipid-based components in the extracted biosurfactant sample. Thio *et al.* (2022) reported that the detection of β -hydroxy fatty acid chains (C10-C12) is characteristic of rhamnolipid biosurfactants. Smyth *et al.* (2010) stated that GC-

MS analysis mainly detects lipid fragments rather than complete biosurfactant molecules. Tawila *et al.* (2022) also reported that the sugar moiety of rhamnolipids is not directly detected in GC-MS due to its non-volatile nature. Therefore, correlation of GC-MS, HPLC, and biochemical assay results confirmed the biosurfactant as a glycolipid-type.

Figure-10: GC-MS chromatograms



Conclusion

In increasing use of synthetic surfactant contributes significantly environmental pollution and poses challan to Human and ecosystem health. the present study successfully demonstrated the isolation, optimization, and characterization of biosurfactant produced by *Kocuria marina*, and characterized biosurfactant-producing bacteria from oil-contaminated soil. Primary and secondary screening assays confirmed biosurfactant production, and isolate 14 (10^{-9}) showed higher emulsification and phenol sulphuric acid assay activity among the tested isolates. MALDI-TOF analysis identified the isolate as *Kocuria marina*. Optimization studies showed that *Kocuria marina* produced higher biosurfactant activity at pH 8.5, with E24 values reaching 65% indicating favourable biosurfactant production under alkaline conditions. Glucose supported higher biosurfactant production compared to sodium nitrate. Biosurfactant extraction by acid precipitation produced white precipitates, confirming successful recovery of the compound. Approximately 0.1-0.3gm of crude biosurfactant was obtained from 200 mL of culture supernatant after acid precipitation. HPLC and GC-MS confirmed the biosurfactant as a glycolipid-type rhamnolipid containing β -hydroxy fatty acid chains (C10-C12). The study demonstrates the potential of *Kocuria marina* for biosurfactant production and its possible application in environmental and industrial processes.

Acknowledgments

The authors express their sincere gratitude to the **Indian Institute of Teacher Education, Gandhinagar** for providing the infrastructure and resources that facilitated this study. Special thanks are extended to the **Centre of Research, Indian Institute of Teacher Education** for the financial support through the *Seed Money Scheme*, which enabled the undertaking of this minor research project. This support has been invaluable in initiating and successfully completing the study.

Special thanks to Ms. Sahin Varaiya and the faculty members for their encouragement, guidance, and constructive suggestions throughout the course of the project. Their insights and support have played a significant role in shaping the outcomes of this research.

References

- Azevedo, V. V. C., Santos, A. T. M., Melo, V. M. M., & Müller, M. M. (2024). Rhamnolipids: A biosurfactant for the development of lipid-based nanosystems for food applications. *Comprehensive Reviews in Food Science and Food Safety*, 23, e13252. <https://doi.org/10.1111/1541-4337.13252>
- Banat, I. M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., Smyth, T. J., & Marchant, R. (2010). Microbial biosurfactants production, applications and

- future potential. *Applied Microbiology and Biotechnology*, 87, 427–444. <https://doi.org/10.1007/s00253-010-2589-0> ([doi.org in Bing](#))
- Chithra, S., & Hemashenpagam, N. (2021). Biosurfactant-producing bacteria isolated from oil contaminated soil and its media optimization for enzyme production. *Bioscience Biotechnology Research Communications*, 14(4), 1613–1619.
- Elazzazy, A. M., Abdelmoneim, T. S., & Almaghrabi, O. A. (2015). Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia. *Saudi Journal of Biological Sciences*, 22(4), 466–475. <https://doi.org/10.1016/j.sjbs.2014.11.018>
- Faisal, Z. G., Mahdi, M. S., & Alobaidi, K. H. (2023). Optimization and chemical characterization of biosurfactant produced from a novel *Pseudomonas guguanensis* strain Iraqi ZG.K.M. *International Journal of Microbiology*, 2023, Article 1571991. <https://doi.org/10.1155/2023/1571991>
- Fakruddin, M. (2012). Biosurfactant: Production and application. *Journal of Petroleum & Environmental Biotechnology*, 3(4), 124. <https://doi.org/10.4172/2157-7463.1000124>
- Farias, C. B. B., Almeida, F. C. G., Silva, I. A., Souza, T. C., Meira, H. M., Soares da Silva, C. F., Luna, J. M., Santos, V. A., Converti, A., Banat, I. M., & Sarubbo, L. A. (2021). Production of green surfactants: Market prospects. *Electronic Journal of Biotechnology*, 51, 28–39. <https://doi.org/10.1016/j.ejbt.2021.02.002>
- Fenibo, E. O., Ijoma, G. N., Selvarajan, R., & Chikere, C. B. (2019). Microbial surfactants: The next generation multifunctional biomolecules for applications in the petroleum industry and its associated environmental remediation. *Microorganisms*, 7(11), 581. <https://doi.org/10.3390/microorganisms7110581>
- Jaysree, R. C., Basu, S., Singh, P. P., Ghosal, T., Patra, P. A., Keerthi, Y., & Rajendran, N. (2011). Isolation of biosurfactant producing bacteria from environmental samples. *Pharmacology online*, 3, 1427–1433.
- Kabeil, S. S., et al. (2025). Rhamnolipids bio-production and miscellaneous applications towards green technologies: A literature review. *PeerJ*, 13, e18981. <https://doi.org/10.7717/peerj.18981>
- Kim, S. B., Nedashkovskaya, O. I., Mikhailov, V. V., Han, S. K., Kim, K. O., Rhee, M. S., & Bae, K. S. (2004). *Kocuria marina* sp. nov., a novel actinobacterium isolated from marine sediment. *International Journal of Systematic and Evolutionary Microbiology*, 54(5), 1617–1620. <https://doi.org/10.1099/ijs.0.02742-0>
- Makkar, R. S., Cameotra, S. S., & Banat, I. M. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB Express*, 1, 5. <https://doi.org/10.1186/2191-0855-1-5>
- Nagode, V. S., Cardoza, C., Yasin, H. K. A., Mali, S. N., Tambe, S. M., Roy, P., Singh, K., Goel, A., Amin, P. D., Thorat, B. R., Cruz, J. N., & Pratap, A. P. (2023). Green surfactants (biosurfactants): A petroleum-free substitute for sustainability—Comparison, applications, market, and future prospects. *ACS Omega*, 8(12), 11674–11699. <https://doi.org/10.1021/acsomega.3c00591>
- Nayariseri, A., Singh, P., & Singh, S. K. (2018). Screening, isolation and characterization of biosurfactant producing *Bacillus subtilis* strain ANSKLAB03. *Bioinformation*, 14(6), 304–314. <https://doi.org/10.6026/97320630014304>
- Pradhan, A. K., Rath, A., Pradhan, N., Hazra, R. K., Nayak, R. R., & Kanjilal, S. (2018). Cyclic lipopeptide biosurfactant from *Bacillus tequilensis* exhibits multifarious activity. *3 Biotech*, 8, 261. <https://doi.org/10.1007/s13205-018-1288-x>
- Ribeiro, B. G., Guerra, J. M. C., & Sarubbo, L. A. (2020). Biosurfactants: Production and application prospects in the food industry. *Biotechnology Progress*, 36, e3030. <https://doi.org/10.1002/btpr.3030>

- Saikia, R. R. (2012). Production of biosurfactant by *Pseudomonas aeruginosa* RS29 isolated from crude oil contaminated soil of Upper Assam and its application in recovery of hydrocarbon from refinery sludge (Doctoral thesis). Gauhati University. <http://hdl.handle.net/10603/28275>
- Santos, D. K. F., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). Biosurfactants: Multifunctional biomolecules of the 21st century. *International Journal of Molecular Sciences*, 17(3), 401. <https://doi.org/10.3390/ijms17030401>
- Saravanan, V., & Vijayakumar, S. (2012). Isolation and screening of biosurfactant producing microorganisms from oil contaminated soil. *Journal of Academic and Industrial Research*, 1(5), 264–268.
- Sarubbo, L. A., Silva, M. G. C., Durval, I. J. B., Bezerra, K. G. O., Ribeiro, B. G., Silva, I. A., Twigg, M. S., & Banat, I. M. (2022). Biosurfactants: Production, properties, applications, trends, and general perspectives. *Biochemical Engineering Journal*, 181, 108377. <https://doi.org/10.1016/j.bej.2022.108377>
- Smyth, T. J. P., Perfumo, A., & Marchant, R. (2010). Isolation and analysis of microbial biosurfactants. *Biotechnology Advances*, 28(3), 375–388. <https://doi.org/10.1016/j.biotechadv.2010.02.001>
- Tawila, S. A., El-Meihy, R. M., Youssef, A. M., Zaghloul, R. A., & Abou-Aly, H. E. (2022). Characterization of rhamnolipids produced by *Pseudomonas putida* ON763757 isolated from petroleum contaminated soils. *Egyptian Journal of Chemistry*, 65(12), 1–11. <https://doi.org/10.21608/EJCHEM.2022.148123.6406>
- Thio, C. W., Lim, W. H., Shah, U. K. M., & Phang, L. Y. (2022). Palm kernel fatty acid distillate as substrate for rhamnolipids production using *Pseudomonas* sp. LM19. *Green Chemistry Letters and Reviews*, 15(1), 83–92. <https://doi.org/10.1080/17518253.2021.2023223>
- Venkataraman, S., Rajendran, D. S., & Vaidyanathan, V. K. (2024). An insight into the utilization of microbial biosurfactants pertaining to their industrial applications in the food sector. *Food Science and Biotechnology*, 33(2), 245–273. <https://doi.org/10.1007/s10068-023-01435-6>
- Wang, X., Li, D., Yue, S., Yuan, Z., & Li, S. (2024). A rare mono-rhamnolipid congener efficiently produced by recombinant *Pseudomonas aeruginosa* YM4 via the expression of global transcriptional regulator irrE. *Molecules*, 29(9), 1992. <https://doi.org/10.3390/molecules29091992>
- Zhou, J., Xue, R., Liu, S., Xu, N., Xin, F., Zhang, W., Jiang, M., & Dong, W. (2019). High di-rhamnolipid production using *Pseudomonas aeruginosa* KT1115, separation of mono/di-rhamnolipids, and evaluation of their properties. *Frontiers in Bioengineering and Biotechnology*, 7, 245. <https://doi.org/10.3389/fbioe.2019.00245>

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DOI: [10.22192/ijarbs.2026.13.06.003](https://doi.org/10.22192/ijarbs.2026.13.06.003)

How to cite this article:

Aditi Gamit, Rashmi Bariya and Mehul Dave. (2026). Extraction and characterization of Biosurfactant produced by *Kocuria marina*. Int. J. Adv. Res. Biol. Sci. 13(6): 22-33.
DOI: <https://dx.doi.org/10.22192/ijarbs.2026.13.06.003>