**RESEARCH PAPER**

# Isolation and Molecular Characterization of Marine-Derived Fictibacillus enclensis Strain 5: A Potent Biosurfactant Producer with Antifungal Activity.

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**ABSTRACT**

Marine ecosystems harbor a vast reservoir of microorganisms capable of producing novel bioactive compounds with environmental and agricultural significance. In this study, marine water samples collected from the mid-sea region of Machilipatnam, Andhra Pradesh, were screened for biosurfactant-producing bacteria using enrichment culture techniques with kerosene as the sole carbon source. Ten distinct isolates ($I_1$–$I_{10}$) were obtained and screened through multiple qualitative and quantitative assays. Among them, isolate $I_5$ demonstrated the highest emulsification index (60.6%), maximum biosurfactant yield (340 mg/L), and significant surface tension reduction (from 40 to 25 mN/m within 48 h), highlighting its potent lipopeptide biosurfactant production. Morphological and staining analyses revealed that isolate $I_5$ is a Gram-positive, spore-forming, rod-shaped, and capsulated bacterium. Biochemical tests suggested its affiliation with the genus Fictibacillus, which was confirmed through 16S rRNA gene sequencing and phylogenetic analysis, identifying the strain as Fictibacillus enclensis strain 5 (GenBank Accession No. PQ864798). Antifungal activity assays revealed 55% and 70% inhibition against Macrophomina phaseolina and Sclerotium rolfsii, respectively, indicating its biocontrol potential. FTIR spectral analysis confirmed the presence of functional groups characteristic of cyclic lipopeptides like surfactin and iturin. These findings demonstrate the potential of Fictibacillus enclensis strain 5 as a sustainable biotechnological candidate for biosurfactant production and fungal pathogen control.

**Keywords**: Marine bacteria; Fictibacillus enclensis; Biosurfactant; Lipopeptide; Surface tension; FTIR; Antifungal activity

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## INTRODUCTION

Marine ecosystems, which span more than 70% of the Earth's surface, serve as vital reservoirs of microbial biodiversity, much of which remains insufficiently characterized (Satpute et al., 2010). These environments pose unique challenges such as high pressure, salinity, temperature fluctuations, and low nutrient concentrations. As a result, marine microorganisms have evolved distinctive adaptive mechanisms, leading to the development of unique metabolic capabilities not typically observed in terrestrial species (Subramani & Aalbersberg, 2012). Marine bacteria, in particular, have emerged as promising candidates for producing a wide range of bioactive metabolites including enzymes, antibiotics, pigments, and biosurfactants (Debnath et al., 2021). These bio-products are being increasingly explored for their applications in pharmaceuticals, agriculture, and environmental remediation. Biosurfactants are amphiphilic molecules produced by various microorganisms such as bacteria, yeasts, and fungi. These molecules contain both hydrophilic and hydrophobic domains, enabling them to lower surface and interfacial tensions and to emulsify hydrophobic compounds (Mnif & Ghribi, 2016). Biosurfactants are broadly classified into glycolipids, lipopeptides, phospholipids, fatty acids, and polymeric types, each with distinct structural and functional properties (Kiran et al., 2011). Compared to chemical surfactants, biosurfactants are eco-friendly, biodegradable, and less toxic, making them attractive alternatives in green technologies (Sachdev & Cameotra, 2013). Additionally, they exhibit antimicrobial, antifungal, antiadhesive, and immunomodulatory properties, supporting their use in a wide range of industries including cosmetics, food, agriculture, and medicine (Banat et al., 2014).

Marine-derived biosurfactant producers are particularly attractive due to their resilience in high-salinity and extreme environments, often resulting in biosurfactants with enhanced emulsifying and antimicrobial properties (Rahman et al., 2021). Bacterial genera such as *Bacillus*, *Pseudomonas*, *Halomonas*, and *Alcanivorax* have demonstrated biosurfactant production in marine ecosystems (Yin et al., 2020). For instance, marine *Bacillus subtilis* and *Pseudomonas stutzeri* strains have been found to produce surfactin and rhamnolipids, respectively, which inhibit phytopathogenic fungi like *Fusarium oxysporum* and *Aspergillus flavus* (Das et al., 2014). The genus *Fictibacillus*, particularly *Fictibacillus enclensis*, isolated from Indian marine sediment, has gained attention for its halotolerance and bioactive potential (Glaeser et al., 2013). Recent findings suggest these strains can produce biosurfactants and antimicrobial enzymes under

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hypersaline conditions, offering potential for applications in saline agriculture, petroleum recovery, and antifungal biocontrol (Goswami et al., 2022; Kumar et al., 2020; Ongena & Jacques, 2008).

## METHODOLOGY

### *Enrichment and isolation of marine biosurfactant-producing bacteria*

Marine water samples were aseptically collected from coastal sites and used for enrichment following the method of Dubey and Juwarkar (2001). One milliliter of each sample was inoculated into 250 mL Erlenmeyer flasks containing 99 mL of sterile mineral salts medium (MSM). Kerosene was added at concentrations of 1 mL, 5 mL, and 10 mL as the sole carbon source to favor the growth of hydrocarbon-degrading, biosurfactant-producing bacteria. The flasks were incubated at 37 °C with shaking at 180 rpm for 72 hours under aerobic conditions to selectively enrich efficient lipopeptide-producing strains.

### *Isolation of pure bacterial cultures*

Following enrichment, serial dilutions of the cultures were prepared up to $10^{-7}$ using sterile distilled water. From selected dilutions, 100 µL aliquots were plated onto nutrient agar using a sterile glass spreader. The plates were incubated at 37 °C for 24–48 hours. Distinct colonies were picked and repeatedly subcultured to obtain pure isolates, which were then maintained on nutrient agar slants at 4 °C for subsequent analyses.

### *Preliminary screening for biosurfactant activity*

Isolated marine bacterial strains were preliminarily screened for biosurfactant production using a series of qualitative assays. These tests targeted various surface-active properties indicative of lipopeptide biosurfactant production, enabling the identification of potential biosurfactant-producing isolates.

**Microplate assay**

Biosurfactant activity was initially screened using a microplate assay. In this assay, 100 µL of culture supernatant was added to wells of a sterile 96-well microtiter plate. The presence of a biosurfactant was indicated by the formation of a thin, uniform film across the well surface, demonstrating reduced surface tension.

**Oil spreading assay**

The oil-spreading assay was used to assess biosurfactant activity. A Petri dish containing 50 mL of distilled water was overlaid with 20 µL of vegetable oil. Then, 10 µL of culture supernatant was added to the center. A clear zone formed by oil displacement indicated positive biosurfactant activity, and the diameter of the zone was measured to evaluate surface activity.

**Penetration method**

The penetration method was applied to assess the ability of biosurfactants to disrupt hydrophobic barriers. A dual-layer agar plate with a paraffin wax top layer was prepared. Drops of cell-free supernatant were placed on the surface, and infiltration or dispersion through the wax after incubation indicated positive biosurfactant activity.

**Emulsification index (E24) assay**

The emulsifying activity was quantified using the Emulsification Index (E24). Equal volumes (2 mL) of culture supernatant and kerosene were mixed in a test tube and vortexed for 2 minutes. After 24 hours of settling at room temperature, the height of the emulsion layer was measured, and E24 was calculated as:

$$E_{24}(\%) = \frac{\text{Height of Emulsion Layer (mm)}}{\text{Total Height of Liquid Column (mm)}} \times 100$$

**Surface tension measurement**

Surface tension was measured using a Du Noüy ring tensiometer. Cell-free supernatants from 24- and 48-hour cultures were collected by centrifugation. After calibrating the instrument with distilled water, measurements were taken at room temperature. The force needed to detach the platinum ring from each sample was recorded. A decrease in surface tension compared to the control indicated biosurfactant production.

**Production and extraction of biosurfactant**

The selected bacterial isolate was grown in 100 mL of sterile MSM in a 500 mL flask at 37 °C and 180 rpm for 48 hours. After incubation, the culture was centrifuged at 10,000 rpm for 15 minutes to collect the supernatant. The pH was adjusted to 2.0 with 6 N HCl, and the mixture was kept at 4 °C overnight for biosurfactant precipitation. The precipitate was recovered by centrifugation (10,000 rpm, 20 minutes), dissolved in methanol, and concentrated using a rotary evaporator to obtain semi-purified biosurfactant.

**Identification and characterization of selected isolates**

Marine bacterial isolates were identified using morphological, biochemical, and molecular methods. Gram's staining was performed for initial characterization. Standard biochemical tests, including starch hydrolysis, indole, MR-VP, citrate utilization, catalase, and glucose fermentation, were conducted as per Bergey's Manual. For molecular analysis, genomic DNA was extracted from the most active isolate, and the 16S rRNA gene was amplified using universal primers. The sequence was analyzed via BLAST and a phylogenetic tree was constructed using MEGA 4. The sequence was submitted to NCBI GenBank for accession.

**Evaluation of antifungal activity using dual culture technique**

The antifungal activity of the marine bacterial isolate was evaluated using the dual culture method against *Sclerotium rolfsii* and *Macrophomina phaseolina*. A 5 mm fungal agar plug was placed at the center of a PDA plate, and the bacterial isolate was streaked 2 cm from the edge. Plates were incubated at 30 ± 2°C for five days. Inhibition zones between the bacterial streak and fungal growth were measured in millimeters. Controls without bacteria were included, and all tests were conducted in triplicate.

**Fourier transform infrared (FTIR) spectroscopic analysis of biosurfactant**

FTIR spectroscopy was used to detect functional groups in the extracted biosurfactant. For transmission mode, the dried sample was mixed with KBr and pressed into pellets. For ATR mode, the extract was dissolved in a suitable solvent and applied to the ATR crystal. Spectra were

recorded between 4000 and 400 $cm^{-1}$ at 4 $cm^{-1}$ resolution, averaging multiple scans for clarity.

**Results**

**Isolation, screening, and selection of novel lipopeptide-producing marine bacteria**

Marine water samples from Machilipatnam, Andhra Pradesh, were enriched in MSM with kerosene as the sole carbon source. After 72 hours of incubation, the cultures showed moderate turbidity and visible emulsification, suggesting the presence of active hydrocarbon-degrading, biosurfactant-producing bacteria (Fig. 1).

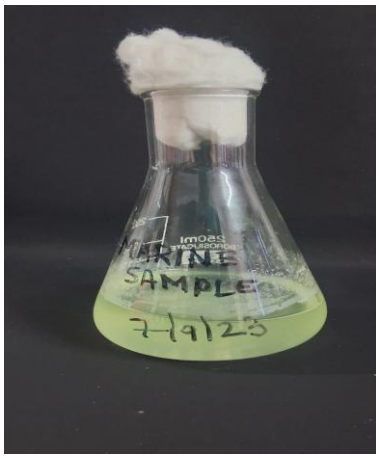



**Figure. 1: Isolation of Biosurfactant producing bacteria by enrichment technique**

Post-enrichment, serial dilution and plating yielded ten distinct bacterial colonies labeled I1-10 (Fig. 2). These isolates exhibited variations in pigmentation, texture, and growth, indicating metabolic diversity. Preliminary qualitative assays revealed that several isolates demonstrated notable emulsifying ability and surface activity, suggesting potential lipopeptide biosurfactant production.

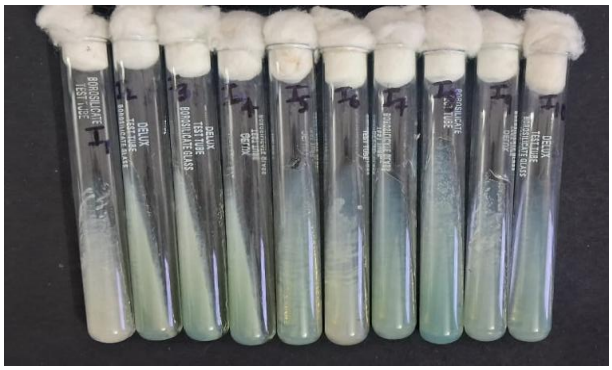


**Figure. 2: Biosurfactant producing bacteria by pure culture method**

**Colony Morphology Studies of Biosurfactant-Producing Bacterial Isolates**

The ten marine bacterial isolates ($I_1$–$I_{10}$) displayed distinct colony morphologies on nutrient agar, varying in color, shape, size, margin, elevation, and opacity. Isolates like $I_1$ and $I_4$ appeared creamy white but differed in elevation and transparency. Similar diversity was noted among other isolates, with differences in colony shape and surface characteristics. These variations helped in differentiating the isolates and selecting phenotypically diverse strains for further analysis (Table. 1).

**Table 1. Colony morphology characteristics of biosurfactant-producing marine bacterial isolates**

| Isolates | Colour | Margin | Size | Shape | Elevation | Opaque |
|---|---|---|---|---|---|---|
| $I_1$ | Creamy white | Entire | Medium | Circular | Flat | Transparent |
| $I_2$ | White | Undulate | Medium | Irregular | Raised | Transparent |
| $I_3$ | White | Entire | Small | Circular | Convex | Non transparent |
| $I_4$ | Creamy white | Entire | Small | Irregular | Raised | Non transparent |
| $I_5$ | White | Entire | Small | Circular | Flat | Transparent |
| $I_6$ | White | Undulate | Medium | Irregular | Raised | Non transparent |
| $I_7$ | White | Undulate | Small | Irregular | Raised | Transparent |
| $I_8$ | White | Undulate | Small | Irregular | Raised | Non transparent |
| $I_9$ | White | Entire | Small | Circular | Flat | Transparent |
| $I_{10}$ | White | Undulate | Medium | Irregular | Flat | Transparent |

**Preliminary screening for biosurfactant activity**

**Microplate method**

All ten isolates were screened for biosurfactant activity using the microplate assay. Varying degrees of concave meniscus formation were observed, indicating differences in surface tension reduction. Isolate $I_5$ showed the most pronounced concavity, suggesting strong biosurfactant production. Moderate activity was noted in $I_2$ and $I_4$, while isolates like $I_1$, $I_6$, and $I_9$ exhibited only slight curvature. These results identified $I_5$ as the most promising strain for further study (Fig. 3).

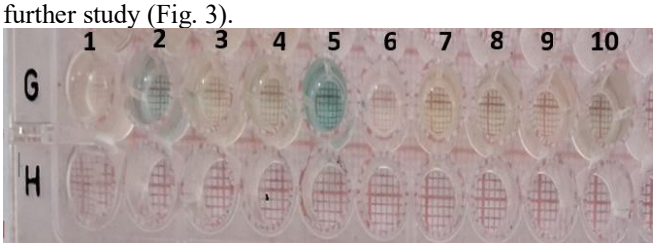



**Figure 3. Microplate-based preliminary screening of biosurfactant activity**

**Biosurfactant screening by penetration method**

The penetration method confirmed biosurfactant activity in all ten isolates through visible color changes in the assay. Isolate $I_5$ exhibited the fastest and most intense shift from red to cloudy white, indicating strong surface activity. Isolates $I_4$, $I_6$, and $I_7$ showed moderate responses, while others displayed weaker or slower transitions. These variations highlight differences in biosurfactant efficiency, with $I_5$ identified as the most effective producer.

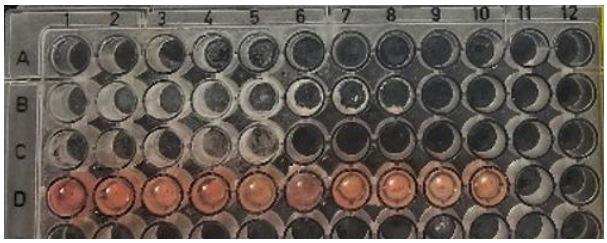



**Figure 4. Penetration assay for biosurfactant screening of marine bacterial isolates**

**Evaluation of biosurfactant activity by oil spread method**

The oil spread assay revealed varying degrees of biosurfactant activity among the ten isolates. Clear zones formed on the oil layer indicated surface activity, with isolate $I_5$ producing the largest zone, reflecting strong emulsification and surface tension reduction. Isolates $I_2$, $I_4$, and $I_6$ showed moderate activity, while others exhibited smaller zones. No clearing was observed in the negative control, confirming the specificity of the response. Isolate $I_5$ emerged as the most efficient biosurfactant producer (Fig. 5).

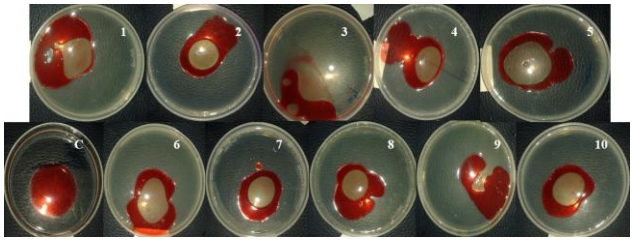



**Figure 5. Oil spread assay of marine bacterial isolates**

**Emulsification index (EI %) assay for biosurfactant activity**

Emulsification index (EI %) analysis revealed differences in emulsifying ability among the ten marine isolates. Isolate $I_5$ exhibited the highest EI at 60.6%, indicating strong biosurfactant activity. Isolates $I_6$ and $I_{10}$ followed with 46.6% and 43.3%, respectively. Isolates $I_4$ and $I_9$ also showed EI values above 40%, while $I_8$ recorded the lowest at 33.3%. These results confirm isolate $I_5$ as the most efficient emulsifier among the tested strains (Fig. 6 & Table. 2).

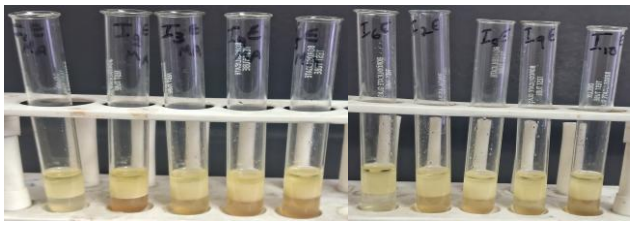


**Figure 6. Emulsification assay of marine isolates**

**Table 2. Emulsification index (EI %) of marine bacterial isolates**

| | Emulsification assays | | Emulsification index (%) |
|---|---|---|---|
| | Total height | Emulsion | |
| $I_1$ | 3cm | 1.2cm | 40% |
| $I_2$ | 3cm | 1.1cm | 36.6% |
| $I_3$ | 3cm | 1.2cm | 40% |
| $I_4$ | 3cm | 1.3cm | 43.3% |
| $I_5$ | 3cm | 1.8cm | 60.6% |
| $I_6$ | 3cm | 1.4cm | 46.6% |
| $I_7$ | 3cm | 1.2cm | 40% |
| $I_8$ | 3cm | 1.0cm | 33.3% |
| $I_9$ | 3cm | 1.2cm | 40% |
| $I_{10}$ | 3cm | 1.3cm | 43.3% |

**Surface tension measurement of culture supernatants**

Surface tension analysis over 48 hours showed a progressive decline in all isolates, indicating active biosurfactant production. Isolate $I_5$ recorded the greatest reduction, dropping from 40 mN/m at 24 hours to 25 mN/m at 48 hours. Isolates $I_6$, $I_9$, and $I_{10}$ also showed values below 30 mN/m at 48 hours (Fig. 7). The control remained unchanged at 60 mN/m, confirming that the surface tension reductions were due to biosurfactant activity.

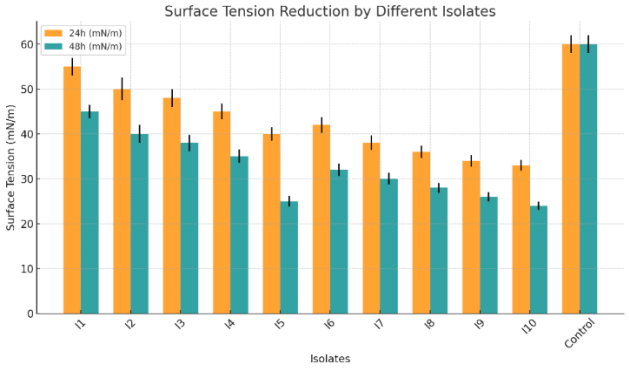



**Figure 7. Surface tension reduction by marine bacterial isolates**

**Quantitative estimation of biosurfactant production**

Quantitative analysis of biosurfactant production after 48 hours revealed notable differences among the marine isolates. Isolate 5 showed the highest yield at 340 mg/L, confirming its strong biosynthetic potential. Isolates 10, 4, and 9 produced between 260–270 mg/L, while moderate levels were noted in isolates 3 and 6. The lowest yield was recorded in isolate 1 at 200 mg/L (Fig. 8). These results align with previous assays, further identifying isolate 5 as the most promising strain.

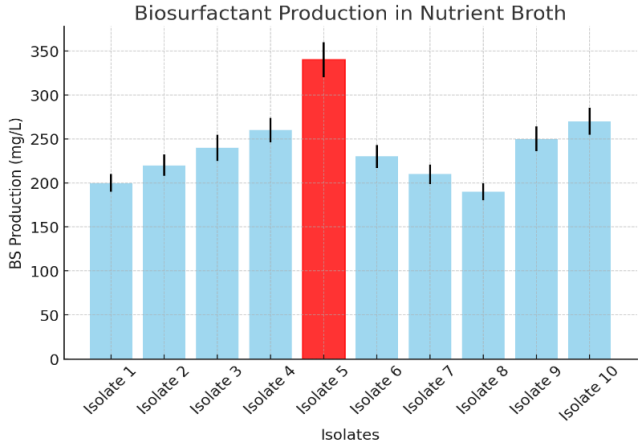



**Figure 8. Quantitative biosurfactant production (mg/L) by marine bacterial isolates**

**Morphological characterization of potent isolate 5**

Morphological staining of isolate 5 confirmed its identity as a Gram-positive, rod-shaped bacterium. Spore staining revealed the presence of endospores, indicating stress tolerance, while capsule staining showed it was capsulated, suggesting enhanced adherence and protection (Table. 3). These features are characteristic of the *Bacillus* genus and support its classification as a robust biosurfactant producer.

**Table 3. Morphological staining characteristics of biosurfactant-producing isolate** 5.

| **Staining** | **Isolate 5** |
|---|---|
| Simple staining | Bacillus |
| Gram's stain | Gram positive |
| Spore stain | Sporulated |
| Capsule stain | Capsulated |

**Biochemical Characterization of Isolate 5**

Biochemical tests of isolate 5 showed positive reactions for methyl red, Voges-Proskauer, citrate utilization, and carbohydrate fermentation, indicating acid production and citrate metabolism. The isolate tested negative for indole, catalase, urease, $H_2S$ production, and starch hydrolysis. Combined with morphological traits, these results are consistent with the characteristics of the *Fictibacillus* genus as described in Bergey's Manual (Figure. 9 & Table. 4).

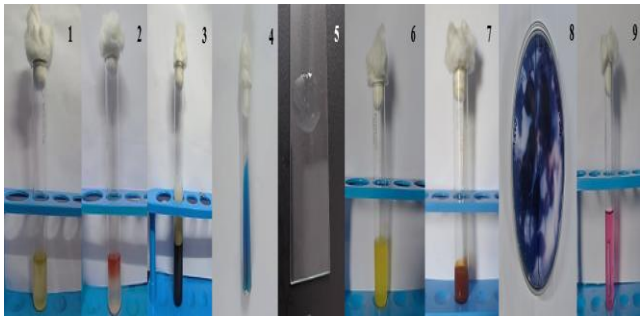



**Figure. 9:(Here 1: Indole test, 2: MR test, 3: VP test. 4: Citrate test, 5: Catalase test, 6: Urease test, 7: H2S test,** 8: Carbohydrate fermentation test and 9: Starch hydrolysis)

**Table 4. Biochemical test results for isolate 5.**

| **Test** | **Result** |
|---|---|
| Indole | Negative |
| Methyl red | Positive |
| Voges proskauer | Positive |
| Citrate | Positive |
| Catalase | Negative |
| Urease | Negative |
| $H_2S$ | Negative |
| Starch hydrolysis | Negative |
| Carbohydrate fermentation | Positive |

**Molecular Identification of Isolate 5**

Molecular identification of isolate 5 via 16S rRNA gene sequencing confirmed its close genetic affiliation with the genus *Fictibacillus*. The sequence was submitted to the NCBI GenBank and assigned the accession number PQ864798.1, identifying the isolate as *Fictibacillus enclensis* strain 5. Phylogenetic analysis using MEGA software positioned isolate 5 close to *Fictibacillus enclensis* strain NIO-1003 with 92% similarity and to *Fictibacillus barbaricus* strain ZT116 with 86% similarity. The tree confirmed its placement within the *Fictibacillus* clade, supporting its identity as a novel strain of *Fictibacillus enclensis (Fig. 10)*. These findings align with earlier morphological and biochemical data, validating its biosurfactant-producing potential.

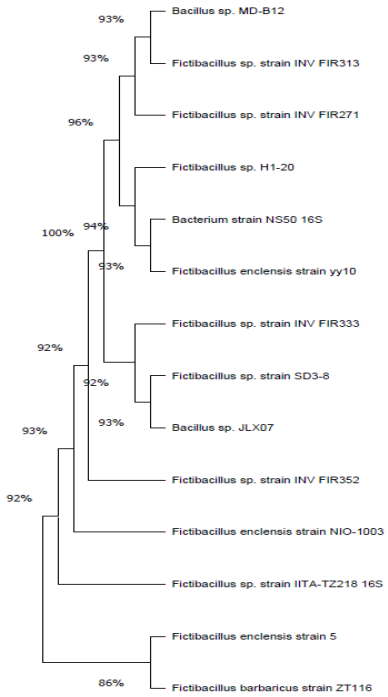



**Figure 10. Phylogenetic tree showing the relationship of *Fictibacillus enclensis* strain 5**

**Antifungal activity of *Fictibacillus enclensis* Strain 5**

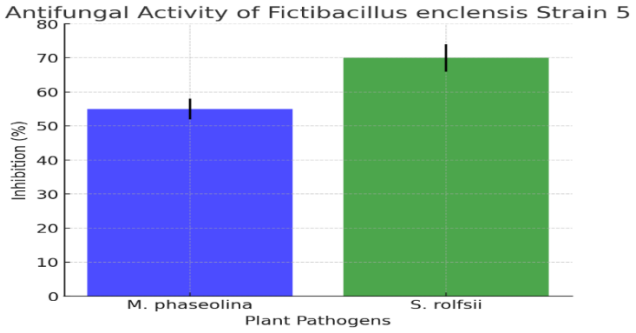
Dual culture assays demonstrated that *Fictibacillus enclensis* strain 5 inhibited the growth of *Macrophomina phaseolina* and *Sclerotium rolfsii*, as evidenced by clear zones of inhibition between the bacterial streak and fungal colonies. These results confirm the strain's strong antifungal potential (Fig.11).

*M. phaseolina* *S. rolfsii*

**Figure 11. Dual culture assay showing the antifungal activity of *Fictibacillus enclensis* strain 5**

Quantitative assessment showed that isolate 5 inhibited *M. phaseolina* by 55%, indicating moderate antifungal activity, while a stronger inhibition of 70% was observed against *S. rolfsii*. These results suggest that the strain's biosurfactant contributes to fungal suppression, likely by disrupting cell membranes or affecting fungal metabolism.

**Figure 12. Percentage inhibition of fungal growth by *Fictibacillus enclensis* strain 5**




**FTIR characterization of biosurfactant extract**

FTIR analysis of the biosurfactant from *Fictibacillus enclensis* strain 5 showed characteristic peaks confirming its lipopeptide nature. A strong band at 1710 $cm^{-1}$ indicated ester-linked carbonyl groups, while the 1527 $cm^{-1}$ peak reflected N–H bending, confirming peptide bonds. Peaks between 2832–2873 $cm^{-1}$ corresponded to C–H stretching in aliphatic chains, validating the lipid component. Additional bands at 1410, 1282, and 1103 $cm^{-1}$ indicated –OH and –CH vibrations, and peaks in the fingerprint region supported the biosurfactant's complex structure (Fig. 13 & Table. 6).

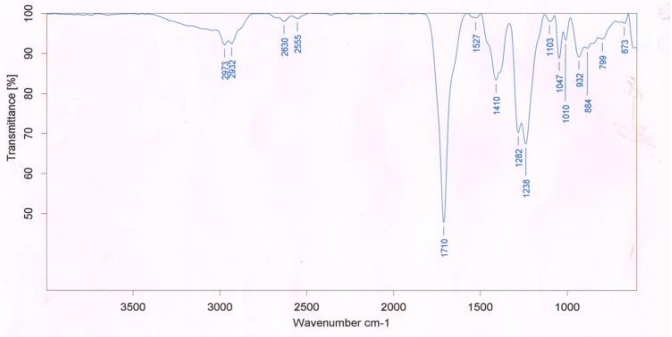



**Figure 13. FTIR spectrum of biosurfactant extracted from *Fictibacillus enclensis* strain 5**

**Table 6. FTIR absorption bands and corresponding functional groups detected in the biosurfactant extract of *Fictibacillus enclensis* strain 5.**

| Peak ($cm^{-1}$) | Functional Group/Stretch | Associated Biosurfactant | Assignment |
|---|---|---|---|
| 3283 – 3282 | O–H stretching (broad) | Iturin and Surfactin | Indicates hydroxyl groups in lipopeptides |
| 2930 – 2923 | C–H stretching (alkanes) | Iturin and Surfactin | Aliphatic hydrocarbon chains |
| 2360 – 2555 | N–H stretching (amide group) | Iturin | Amide bond in peptide linkage |
| 1710 | C=O stretching (ester/lactone group) | Surfactin | Lactone ring system |

| | | | |
|---|---|---|---|
| 1527 | N–H bending (secondary amide group) | Iturin | Confirms peptide linkage in cyclic lipopeptides |
| 1410 | C–H bending | Iturin and Surfactin | Aliphatic chain vibrations |
| 1282 – 1283 | C–O stretching (ester group) | Surfactin | Lactone group confirmation |
| 1103 – 1047 | C–O–C stretching (ether group) | Iturin | Indicates ether linkages |
| 1010 | C–C stretching (alkane) | Iturin and Surfactin | Aliphatic chain confirmation |
| 932 | C–H bending | Iturin and Surfactin | Aliphatic hydrocarbon chains |
| 884 | C=C bending | Surfactin | Confirms cyclic peptide structure |
| 789 | C–H wagging (aromatic) | Iturin | Aromatic ring confirmation in peptides |
| 673 | C–H out-of-plane bending | Iturin and Surfactin | Hydrocarbon chain confirmation |

## DISCUSSION

The current study highlights the successful isolation and characterization of biosurfactant-producing marine bacteria from the Machilipatnam mid-sea region, revealing the biotechnological potential of these environmental isolates. The use of kerosene-enriched mineral salt medium (MSM) effectively selected for hydrocarbon-degrading microbes, leading to the isolation of ten phenotypically diverse strains ($I_1$–$I_{10}$). Consistent with Kumar et al. (2020), such enrichment protocols favor metabolically adaptive bacteria capable of biosurfactant synthesis under hydrophobic conditions. Morphological observations revealed significant variation among the isolates, with differences in pigmentation, elevation, and margin types, reflecting underlying functional diversity. Traits observed in isolates like $I_1$ and $I_2$ aligned with previously reported biosurfactant-producing Bacillus and Pseudomonas species (Thavasi et al., 2011; Suresh et al., 2023). Transparent colony features noted in several isolates further supported their potential biosurfactant activity, as seen in earlier studies by Ramakrishna et al. (2022).

The microplate and penetration assays effectively screened for biosurfactant activity, with isolate $I_5$ consistently demonstrating the strongest response in both methods. This confirms its ability to reduce surface tension and emulsify hydrophobic compounds, aligning with results from Saimmai et al. (2012) and Satpute et al. (2010). The oil spread method further validated this observation, as $I_5$ exhibited the largest oil displacement zone, similar to findings by Haddad et al. (2009). Quantitative assays reinforced these findings. The emulsification index (EI%) ranged widely, with isolate $I_5$ achieving the highest EI of 60.6%, indicating strong emulsifying potential comparable to marine biosurfactant producers described by Thavasi et al. (2011) and Cameotra & Makkar (2004). Surface tension reduction from 40 to 25 mN/m by isolate $I_5$ at 48 hours demonstrates potent surfactant activity, consistent with reports on Bacillus spp. by Joshi et al. (2008) and Das et al. (2008). Biosurfactant yield analysis confirmed isolate $I_5$ as a high producer, yielding 340 mg/L, which falls within the range reported for Bacillus and Pseudomonas species (Mulligan, 2005; Chen et al., 2020). Other isolates such as $I_{10}$ and $I_9$ also showed substantial production, indicating their suitability for further exploration.

Morphological and biochemical traits of isolate $I_5$, including its Gram-positive, rod-shaped, endospore-forming nature and capsule production, place it within the genus *Fictibacillus*. These characteristics agree with descriptions by Logan & De Vos (2009) and Stewart (2012). The biochemical fingerprint matches closely with *Fictibacillus enclensis* traits noted by Glaeser et al. (2013) and Goswami et al. (2022). Molecular identification through 16S rRNA sequencing confirmed its identity as *F. enclensis* with a GenBank accession number PQ864798.1 and <95% similarity, suggesting a potential novel variant. Antagonistic studies using dual culture assays revealed strong antifungal activity of *F. enclensis* strain 5 against *Macrophomina phaseolina* (55% inhibition) and *Sclerotium rolfsii* (70% inhibition), highlighting its biocontrol potential. This corresponds with the mechanisms proposed by Vijayakumar & Saravanan (2015) and the known actions of lipopeptides like iturin and surfactin (Ongena & Jacques, 2008; Arrebola et al., 2010).

FTIR spectral analysis confirmed the presence of lipopeptide structures in the biosurfactant extract, with characteristic peaks representing ester, peptide, and aliphatic groups (Mukherjee et al., 2006; Smyth et al., 2010). These spectra resembled those of surfactin-like compounds previously reported by Jasim et al. (2016), further supporting the identification of a stable, multifunctional biosurfactant with broad application potential in environmental and industrial biotechnology (Gautam & Tyagi, 2006).

## CONCLUSION

The present study successfully identified *Fictibacillus enclensis* strain 5 as a novel marine-derived bacterium with significant biosurfactant-producing and antifungal capabilities. Through systematic isolation, morphological, biochemical, molecular, and functional screening, isolate 5 emerged as a potent candidate with high emulsification index, strong surface tension reduction, and remarkable biosurfactant yield. Its inhibitory activity against

*Macrophomina phaseolina* and *Sclerotium rolfsii* further highlights its potential as a biological control agent. FTIR analysis confirmed the presence of lipopeptide functional groups, underscoring its biochemical nature. Collectively, these results position *F. enclensis* strain 5 as a promising bioresource for eco-friendly applications in agriculture and environmental remediation.

**Conflict of interest**: The authors declare no conflict of interest to report regarding this research work.

**Data Availability**: All data generated or analyzed during this study are included in this article.

**Ethical approval**: The authors confirm that there are no ethical issues in the publication of the manuscript.

**Human and animal rights**: No animals/humans were used for studies that are the basis of this research.

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