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Deep sea coral parrot fish mucus: A novel reservoir of marine biosurfactant

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Abstract

Biosurfactants are eco-friendly surface-active biomolecules with significant industrial and biomedical applications. Marine host-associated microhabitats remain largely unexplored for novel biosurfactant-producing microorganisms. Parrot fish mucus, which serves as a protective barrier, harbors a diverse microbial community with unique metabolic capabilities, making it a promising source for bioprospecting. In this study, bacteria were isolated from parrot fish mucus and screened for biosurfactant production. A total of eleven isolates were obtained and subjected to a three-stage evaluation process. Primary screening involved oil displacement and hemolytic assays, followed by secondary screening using emulsification index (E24) and surface tension reduction tests. Several isolates exhibited notable biosurfactant activity. One potent strain demonstrated superior performance, showing significant oil displacement, high emulsification activity, marked hemolysis, and effective surface tension reduction. The selected isolate was further assessed for stability under varying pH, temperature, and salinity conditions. The biosurfactant retained considerable activity across a broad range of environmental parameters, indicating strong stability and suitability for marine applications. Preliminary characterization suggested the presence of amphiphilic compounds, likely glycolipids or lipopeptides. These findings highlight parrot fish mucus as a valuable niche for sustainable biosurfactant-producing bacteria with potential applications in bioremediation, pharmaceuticals, and marine biotechnology.

Keywords: Biosurfactant; Biotechnology; Environmental; Fish and Marine

1. Introduction

Biosurfactants are surface-active biomolecules produced by a wide range of microorganisms, capable of reducing surface and interfacial tension due to their amphiphilic structure (Zajic et al., 1983). Structurally, they include glycolipids, lipopeptides, phospholipids, polymeric surfactants, and fatty acids, each exhibiting distinct physicochemical and biological properties. Compared to synthetic surfactants, biosurfactants possess several ecological and functional advantages such as biodegradability, low toxicity, high specificity, and stability over a wide range of salinity, temperature, and pH conditions (Jeong et al., 2026; Fernandes et al., 2023). These features make them particularly attractive for applications in environmentally sensitive sectors. Due to these favorable properties, biosurfactants have gained increasing attention in diverse industrial and biotechnological fields, including environmental remediation (oil spill recovery and heavy metal removal), pharmaceuticals (drug delivery, antimicrobial and antiadhesive agents), food processing (emulsifiers and preservatives), cosmetics, and marine biotechnology (Datta and Bhattacharjee, 2026; Morin-Crini et al., 2019). However, large-scale commercial utilization remains limited by high production costs and the restricted availability of efficient biosurfactant-producing strains. This has intensified the search for novel microorganisms capable of producing high-yield, structurally unique, and functionally robust biosurfactants. Natural and underexplored ecosystems have emerged as promising reservoirs for discovering new biosurfactant-producing microorganisms (Jackson et al., 2015). Among these, marine environments are particularly valuable due to their immense microbial diversity shaped by extreme and fluctuating conditions such as high salinity, pressure variations,

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ultraviolet radiation, and nutrient scarcity. Microorganisms adapted to such environments often possess specialized metabolic pathways, leading to the synthesis of chemically diverse and stress-tolerant biomolecules with enhanced bioactivity (Wani et al., 2022).

Within marine ecosystems, host-associated microhabitats represent an important yet underinvestigated niche for microbial bioprospecting. The mucus layer of fish acts as a dynamic interface between the host and its environment, serving both protective and ecological functions (Benhamed et al., 2014). Fish mucus contains a complex mixture of glycoproteins, lipids, enzymes, antimicrobial peptides, and organic nutrients, creating a favorable environment for the establishment of a distinct and metabolically active microbial community (Gómez et al., 2013). These mucus-associated microorganisms are continuously exposed to host immune factors, microbial competition, and environmental stressors, conditions that may stimulate the production of secondary metabolites, including biosurfactants (De Carvalho and Fernandes, 2010). Parrot fish mucus, in particular, is continuously secreted and enriched with organic compounds derived from diet and metabolic processes, making it a nutrient-rich microenvironment for microbial colonization (Nazurally et al., 2023). The ecological role of parrot fish in coral reef systems further suggests that their associated microbiota may possess unique functional traits linked to surface colonization, biofilm formation, and antimicrobial defense. Despite this potential, fish mucus-associated microbial communities remain poorly explored, especially in comparison to free-living marine bacteria, with limited studies addressing their capacity for biosurfactant production (Surovy, 2020). In this context, the present study aims to isolate and screen biosurfactant-producing bacteria from the mucus of parrot fish. By exploring this underexplored marine microhabitat, the study seeks to identify novel bacterial strains with potential biotechnological applications and to contribute to a better understanding of fish-associated microbial resources as promising candidates for sustainable biosurfactant production.

2. Materials and Methods

2.1. Sample Collection

Parrot fish were collected from the cuddalore port. Immediately after collection, the fish were placed in sterile polyethylene bags, stored on ice, and transported to the laboratory within 4 h to prevent microbial alteration. External mucus was aseptically collected from the body surface using sterile cotton swabs.

2.2. Isolation

Mucus samples were vortexed thoroughly to detach associated microorganisms. Serial dilutions (10^{-1} to 10^{-6}) were prepared using sterile. Aliquots (100 µL) from appropriate dilutions were spread onto Zobell Marine Agar plates. The plates were incubated at 30 ± 2 °C for 24–48 h. Morphologically distinct colonies were selected and purified by repeated streaking to obtain axenic cultures. Pure isolates were maintained on marine agar slant and preserved in glycerol stocks (20%, v/v).

2.3. Preparation of Cell-Free Culture Supernatant

Each bacterial isolate was inoculated into marine broth and incubated at 30 °C with shaking at 150 rpm for 48–72 h. After incubation, the cultures were centrifuged at 8,000–10,000 rpm for 15 min to remove bacterial cells. The resulting cell-free supernatant was collected and used for biosurfactant screening assays.

2.4. Hemolytic Activity Assay

Hemolytic activity was assessed as a preliminary indicator of biosurfactant production. Bacterial isolates were streaked onto blood agar plates containing 5% (v/v) defibrinated blood and incubated at 30 °C for 24 h. The formation of clear or partially clear zones around colonies was recorded as positive hemolytic activity. (Arifiyanto, A., et al . 2020).

2.5. Oil Displacement Assay

The oil displacement test was performed to evaluate surface activity. Distilled water (40 mL) was added to a Petri dish, followed by the addition of a thin layer of crude oil. A small volume (20 µL) of culture supernatant was gently placed at the center of the oil layer. The diameter of the clear zone formed due to oil displacement was measured in centimeters, indicating biosurfactant activity. (Chakraborty, S., et al. 2015).

2.6. Emulsification Index (E24)

The emulsification ability of the culture supernatant was determined using the emulsification index. Equal volumes of cell-free supernatant and hydrophobic substrate diesel, kerosene and crude oil) were mixed vigorously for 2 min and

allowed to stand undisturbed for 24 h. The height of the emulsion layer was measured and expressed as a percentage of the total liquid column height (E24).

2.7. Drop Collapse Assay

The drop collapse test was carried out on a hydrophobic surface to assess surfactant activity. A drop of oil was placed on a clean glass slide, and a small volume of culture supernatant was added to the oil drop. Flattening or spreading of the drop indicated the presence of biosurfactant, while intact drops were considered negative.

2.8. Surface Tension Measurement

Surface tension of the culture supernatant from selected isolates was measured using a tensiometer. Distilled water served as the control. A significant reduction in surface tension compared to the control was considered confirmation of biosurfactant production.

2.9. Selection of Potent Biosurfactant-Producing Isolates

Isolates showing positive results in multiple screening assays were considered potential biosurfactant producers. The most efficient isolate was selected based on maximum oil displacement, emulsification index, and surface tension reduction for further production and characterization studies.

2.10. Identification of the isolates

Biochemical identification in order to identify and characterize the bacteria isolates, biochemical tests was carried out such as: Gram staining, oxidation/fermentation, production of acid from carbohydrates, hydrolysis of gelatin and citrate. These tests done according to the Bergey's manual for identification taxonomy (Holt et al., 1999)

3. Result

3.1. Biosurfactant production by isolated strains

Eleven isolated strains that show high growth rate on diesel oil were selected for further study. Table 1 illustrates the results of screening tests for these isolated strains. As shown in this table the best isolates that have sufficient response to all screening tests were ST2, ST3, ST5, ST6, ST6, ST7, ST8, ST9, ST10 and ST 11. However isolated strains such as, and have positive response to one or two screening tests (Table 1).

3.2. Biochemical properties of the isolated strains

Identification tests were done to discriminate between isolated strains. Table 2 shows the results of these diag nostic tests. As shown in this table the most isolated strains were Gram negative, oxidase positive and motility positive. However, there are differences between isolated bacteria (Table 2).

3.3. Cell surface hydrophobicity, emulsification index and decrease of surface tension by isolated strains

Emulsification activity, bacterial adhesion to hydrocarbon (BATH) and reduction of surface tension assay were analyzed for each strain separately. Data obtained from these tests were shown in Table 3. Strains and have theST1, ST2, ST3, ST5, ST6, ST7, ST8, ST10, ST11 highest values of emulsification activity and cell surface hydropho bicity. These strains also produce more biosurfactant and ST9 decrease surface tension than other strains. The results indicated that there is a direct relationship between cell surface hydrophobicity and biosurfactant production with emulsification activity (Table 3).

3.4. Molecular identification and phylogenetic analysis of isolated strains

Molecular identification of the isolates was performed for ST1, ST2, ST3, ST4, ST5, ST6, ST7, ST8, ST9, ST10 and ST11 strains by amplification and sequencing the 16S rRNA gene sequencing and comparing them to the database of known 16S rRNA sequences. The molecular identification of these strains show that these strains belong to Shewanella alga (Strain 1), Shewanella upenei (Strain 2), Vibrio furnissii (Strain 3), Gallaecimonas pentaromativorans (Strain 4), Brevibacterium epidermidis (Strain 5), Psychrobacter namhaensis (Strain 6) and Pseudomonas fluorescens (Strain 7). The phylogeny tree of these isolated strains All sequences of seven bacteria were submitted to Genetic sequence database at the National Center for Biotechnical Information (NCBI). Gene Bank ID of these strains in NCBI is: HF781632.

Table 1 Biosurfactant production assay of isolated strain

Isolate	Haemolytic test	Drop collapse (shape of drop in comparison to blank)	Oil spreading (area of displaced in mm)
ST1	+	++	12
ST2	+	+++	10
ST3	-	+++	14
ST4	-	+++	9.5
ST5	+	++	10
ST6	-	+++	11
ST7	+	+++	10
ST8	+	++	10
ST9	+	+++	11
ST10	-	+	6.9
ST11	-	+++	11

Abbreviations: haemolytic test + positive response, - negative response; + low flat, ++middle flat, +++ completely flat (for drop collapse test).

Table 2 Biochemical characteristion of selected biosurfactant producing strains

Biochemical Test	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST11
Gram reaction	+	+	–	+	–	+	+	–	+	–	+
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Catalase	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	–	+	+	–	+	+	–	+	+
Indole	–	–	–	–	–	–	–	–	–	–	–
Methyl red	+	–	+	+	–	+	–	+	+	–	+
Voges–Proskauer	–	+	–	–	+	–	+	–	–	+	–
Citrate utilization	+	+	–	+	–	+	+	–	+	–	+
Urease	–	–	–	–	–	–	–	–	–	–	–
Nitrate reduction	+	+	–	–	+	–	+	–	+	+	–
Glucose fermentation	+	+	+	+	+	+	+	+	+	+	+
Lactose fermentation	–	–	+	–	–	+	–	+	–	–	+
Sucrose fermentation	+	+	–	+	–	–	+	–	+	+	–

Abbreviation: + positive – negative

Table 3 Measurement of emulsification activity (%, E24), cell surface hydrophobicity (%, BATH) and decrease of surface tension (mN/m) by strains in this study. all data that reported in this table is the mean of three values and cited value in the table is standard deviation

Isolate Strains	Emulsification Activity (%, E24),	Bath Assay (%, Bath)	Surface Tension (Mn/M)
ST1	35	26	42.5
ST2	64	58	34.2
ST3	46	28	44.5
ST4	44	20	45
ST5	20	6.8	46
ST6	34	5.2	47
ST7	12	38.8	48
ST8	25	39	50.2
ST9	40	21	47.8
ST10	20	4.7	50.2
ST11	25	1.7	49.2

Surface tension of ONR medium without bacteria as blank 51.6

4. Discussion

Biosurfactants can ful fill various physiological roles and provide ecological advantages to the producing strains (Rosenberg, E., and Ron, E. Z. (1999). For instance, they improve the bioavailability of water insoluble substrates by emulsification, thus enhancing biodegradation of several environmental pollutants. Biosurfactant-producing microbes can be found in various ecosystems, although the environments that are impacted with hydrophobic contaminants such as refinery wastes and petroleum are more yielding than undisturbed ones (Batista et al., 2006). Emulsification of the diesel oil in water is a prerequisite that paves the way for biodegradation of this environmental pollutant by many bacteria. It enhances the bioavailability of the oil and thus increases the biodegradation rate (Gidudu, B., and Chirwa, E. M. (2023) Hassanshahian et al., 2012). However, visual inspection of the diesel oil cultures for biosurfactant production is an indirect screening method as the growth with hydrophobic compounds indicates the production of biosurfactants, but does not always correlate with this trait (Willumsen, P. A., and Karlson, U. (1996).). Second screening phase to further investigate biosurfactant production contain three tests: oil spreading, drop collapse and hemolytic activity assays. The clear zone diameters were larger for cultures of the strains ST 2 ST3 ST4 ST5 ST8 ST9 however the largest diameters observed for strain ST 10 Thus, indi cating the presence of higher concentrations of biosurfactant. This is in good agreement with the drop collapse and hemolytic activity results. Microbial production of the surface-active compounds on diesel oil and other hydrophobic substrates has frequently been reported (Kumara et al., 2006). The data of the third screening phase (surface tension reduction and emulsification activity) confirmed unequivocally the production of biosurfactants by the mentioned strains. Taken together, the data of the screening assays in the three phases are coherent and support each other. Since all the screening assays were per formed with cell-free culture supernatants, it can be concluded that the cells produce biosurfactant and secrete them to the extra cellular medium. Similar results have been reported in many cases. (Lotfabad, T. B., et al .2009).

5. Conclusion

Biosurfactant Producing Marine Microorganisms from Parrot Fish at Cuddalore Port. In this survey from 11 biosurfactant producing bacteria, 7 strains can produce high biosurfactant and reduce surface tension. Also, we found a direct relationship between cell surface hydrophobicity and biosurfactant production with emulsification activity as the strains ST 10 that had high biosurfactant production also had efficient emulsification activity.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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