RESEARCH PAPER

Screening And Evaluation Of Marine Bacteria For Siderophore Production From The Maharashtra Coast, India

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Abstract:
Iron limitation is a defining characteristic of marine environments, necessitating siderophore-mediated iron acquisition by microorganisms. The present study aimed to isolate, screen, and characterize siderophore-producing marine bacteria from coastal ecosystems of Maharashtra, India. Marine water and sediment samples were collected from multiple coastal and estuarine locations and processed using marine-specific enrichment and isolation methods. A total of 55 morphologically distinct bacterial isolates were obtained and screened for siderophore production using qualitative Chrome Azurol S (CAS) broth and agar assays, followed by quantitative estimation using the CAS shuttle assay. Twenty-two isolates (40.0%) exhibited rapid siderophore production in liquid CAS assays, while 13 isolates (23.6%) demonstrated siderophore activity on solid medium. Quantitative analysis revealed substantial inter-strain variability, with siderophore chelation efficiencies ranging from 4.6% to 64.3%. Isolates WS1 and GSW13 were identified as the most efficient producers. Siderophores extracted using ethyl acetate yielded 90–120 mg of secondary metabolites and retained iron-chelating activity following extraction. Molecular identification based on 16S rRNA gene sequencing identified WS1 as *Pseudomonas lundensis* and GSW13 as *Advenella mimigardefordensis*. These findings provide a foundational basis for further exploration of marine-derived siderophores in pharmaceutical research, particularly in iron-chelation systems and siderophore-enabled drug delivery strategies.

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Introduction:

Iron is an essential micronutrient required for core cellular functions, including respiration, electron transport, and DNA synthesis (Andrews et al., 2013). Yet, in marine environments, iron predominantly exists as insoluble ferric oxides, resulting in extremely low bioavailability and imposing a major ecological constraint on microbial growth (Boyd & Ellwood, 2010). To counter this limitation, marine microorganisms synthesize siderophores—high-affinity iron-chelating molecules that solubilize and mobilize Fe^{3+}, enabling survival in iron-depleted niches (Miethke & Marahiel, 2007; Neilands, 1995).

Marine siderophores exhibit exceptional chemical diversity, including amphiphilic backbones, halogenated moieties, and photoreactive functional groups, reflecting evolutionary adaptation to seawater chemistry (Martinez et al., 2017). Beyond facilitating iron uptake, these metabolites shape microbial interactions, influence community structure, and contribute to iron cycling, thereby affecting broader biogeochemical processes (Kraemer et al., 2015). Their ecological relevance has fuelled growing interest in their biotechnological potential, spanning pharmaceuticals, agriculture, bioremediation, and metallurgical applications (Compant et al., 2005; Glick, 2014).

Given their structural novelty and functional versatility, exploring siderophore-producing marine microorganisms is critical for both fundamental understanding and applied innovation.

Accordingly, the present study focuses on:

(i) systematic sampling of marine resources along the Maharashtra coastline,
(ii) isolation and screening of bacterial strains for siderophore production, and
(iii) identification of the most efficient siderophore producers through combined qualitative (CAS agar/broth) and quantitative (CAS-shuttle assay) analyses.

Materials and Methods:

Sample Collection

Marine samples were collected from multiple coastal and estuarine locations along the Maharashtra coastline, including Gateway of India, Elephanta Island, Gorai, Nerul, Girgaon, and Thane Creek, with additional sampling carried out along the Konkan coastline at Kalbadevi, Ware Beach, Malgund, Ganpatipule, Jaigad, Undi, Devghali, Gaonkhadi, Sindhudurg, and Tarkarli.

Sampling included marine water, sand, and mud to capture microbial diversity across varied ecological habitats. Water samples were collected using sterile 500 mL polypropylene bottles submerged 15–30 cm below the surface. Sediment samples were aseptically transferred into sterile polythene bags. In situ measurements of pH and temperature were recorded immediately.

All samples were transported to the laboratory in insulated containers with ice packs to preserve viability.

Enrichment and Isolation

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Enrichment of marine microorganisms was carried out using St. Zobell Marine Broth. Ten millilitres of water sample or 1 g of sediment was inoculated into 90 mL broth and incubated at 28–30°C for 48 hours at 120 rpm. Following enrichment, cultures were streaked onto St. Zobell Marine Agar plates and incubated at 28–30°C for 24–72 hours. Distinct colonies were repeatedly sub-cultured to obtain pure isolates. A total of 55 morphologically distinct isolates were recovered from all sampling locations combined. All isolates were maintained on marine agar slants at 4°C.

Screening for Siderophore Production

Qualitative CAS Broth and Plate Assays

Siderophore production was screened using the Chrome Azurol Sulfonate (CAS) assay following the universal method described by Schwyn and Neilands (1987), with modifications suggested by Louden et al. (2011). CAS agar containing the blue CAS–Fe^{3+} complex was spot-inoculated and incubated at 37°C for 24–48 hours. Siderophore production resulted in a blue-to-yellow/orange color change due to ferric ion removal. Halo diameter indicated siderophore activity. Several isolates showed rapid color change in broth assays and distinct halos on CAS agar plates. *Bacillus subtilis* was used as the positive control.

Quantitative Siderophore Estimation (CAS Shuttle Assay)

Quantitative estimation of siderophore production was carried out using the CAS shuttle assay following Schwyn and Neilands (1987) and the modified microplate-based method of Arora and Verma (2011). Cultures were grown in iron-deficient minimal medium at 37°C for 24 hours under static and shaking conditions (100 rpm). After centrifugation at 3000 rpm for 15 minutes, 0.5 mL of supernatant was mixed with 0.5 mL CAS reagent and 10 µL sulfosalicylic acid shuttle solution. After 20 minutes, absorbance was measured at 630 nm. Siderophore production efficiency was calculated using:

Efficiency (%) = $\{(Ar - As) / Ar\} \times 100$

Where:

Ar = absorbance of reference

As = absorbance of sample

Extraction of Siderophores

High-producing isolates were inoculated into 100 mL St. Zobell Marine Broth and incubated for 72 hours at 30°C. Cultures were centrifuged at 10,000 rpm for 15 minutes. Equal volumes of ethyl acetate were added, mixed, and allowed to separate in a separatory funnel. The organic layer was evaporated at 40°C to obtain crude siderophore extract, which was weighed and reconstituted for further testing as described by Rai et al. (2016).

Testing of Siderophore Activity (CAS Agar Cup Method)

CAS agar plates were prepared and wells were cut using a sterile cork borer. Each well received 50 µL of the extracted siderophore solution. Plates were incubated at 30°C for 24 hours as mentioned by Patel et al. (2016). A blue-to-orange zone around the wells indicated iron chelation activity, with zone diameter corresponding to chelation strength.

Molecular Identification of Selected Isolates

The two most efficient siderophore-producing isolates, WS1 and GSW13, were identified using 16S rRNA gene sequencing. Genomic DNA was extracted, and the 16S rRNA gene was amplified using universal bacterial primers. Sequencing results were analyzed using BLAST against the NCBI GenBank database.

Results and Discussion:

Sampling Coverage and Environmental Parameters

Sampling was conducted across major coastal zones of Maharashtra, including , Gateway of India, Elephanta Island, Gorai, Nerul, Girgaon, and Thane Creek, with additional sampling carried out along the Konkan coastline at Kalbadevi, Ware Beach, Malgund, Ganpatipule, Jaigad, Undi, Devghali, Gaonkhadi, Sindhudurg, and Tarkarli.

A total of 25 samples, comprising marine water and sediment (sand and mud), were collected across these regions. Environmental variations were evident, with pH ranging from 6.5 to 8.5 and temperatures between 22°C and 32°C. These physicochemical gradients indicate diverse ecological niches influencing microbial distribution and siderophore biosynthetic potential.

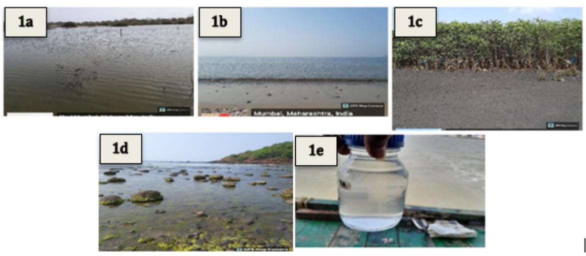

Figure 1- Sampling of marine resources.

1a. Sampling location showing shallow coastal waters with mangrove-associated tidal influence. **1b.** Open sea region depicting clear water column and shoreline characteristics. **1c.** Mangrove-associated sediment zone representing characteristic intertidal mudflat environment.**1d.** Rocky coastal habitat from the southern Konkan belt showing algal and tidal-zone biodiversity.**1e.** Marine water sample collected in sterile polypropylene bottle during field sampling.

Enrichment and Isolation of Marine Bacteria

Enrichment in St. Zobell Marine Broth followed by isolation on St. Zobell Marine Agar yielded 55 morphologically distinct marine bacterial isolates. The isolates varied in pigmentation, surface characteristics, colony elevation, and margin structure, indicating the presence of multiple genera adapted to saline and nutrient-variable marine environments.

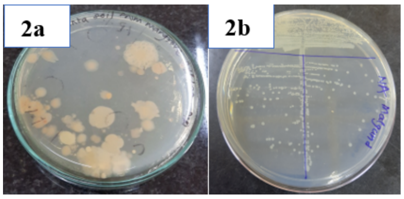

Figure 2. Representative isolation of marine bacteria on Zobell Marine Agar.
2a. Primary colonies following enrichment.
2b. Purified isolates after sub-culturing.

Qualitative Screening for Siderophore Production

A total of 55 marine bacterial isolates were screened qualitatively and quantitatively for siderophore production using the Chrome Azurol S (CAS) assay. The screening revealed considerable heterogeneity among the isolates with respect to response time, intensity of colour change, and iron-chelating potential, indicating marked differences in siderophore-producing ability. During qualitative screening using the CAS broth assay, 22 isolates (40.0%) exhibited a rapid colour transition from blue to orange within 10 minutes, suggesting strong and immediate extracellular siderophore secretion. Further confirmation using the CAS agar plate assay showed that 13 isolates (23.6%) produced distinct orange halos after 24 hours of incubation, reflecting variability in siderophore diffusion and expression under solid-phase conditions. Quantitative estimation using the modified CAS shuttle assay further demonstrated clear differentiation among the siderophore-positive isolates. The comparative iron-chelation efficiencies of representative isolates are summarized in Table 1, enabling classification into high and moderate siderophore producers.

CAS Broth Assay

Using the Chrome Azurol S (CAS) liquid assay, 22 of the 55 tested isolates (40%) demonstrated rapid color transition from blue to orange/yellow, confirming strong extracellular siderophore secretion. Rapid response in isolates such as GSW13, WS1, EMW1and KSW2 suggests efficient iron-chelating capability.

CAS Agar Assay

In CAS agar screening, 13 isolates (23.6%) produced distinct orange halos, indicating siderophore diffusion and activity on solid medium. GSW13 produced the most prominent halo, followed by WS1. The positive control, *Bacillus subtilis*, confirmed assay reliability. The difference observed between liquid and solid-phase assays reflects variability in siderophore expression and diffusibility under different growth conditions.

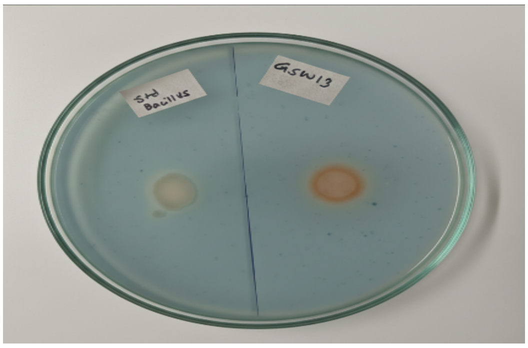

Figure 4. CAS plate assay positive result showing GSW13 and Std. *Bacillus*

Quantitative CAS Shuttle Assay

Quantitative CAS Shuttle Assay revealed siderophore efficiencies ranging from 4.6% to 64.3%, demonstrating substantial variability among isolates. This inter-strain variability suggests that siderophore production is a strain-specific trait rather than a uniform characteristic among marine bacteria. The top five producers were GSW13, WS1, EMW1, KSW2 and TSW1. These isolates exhibit high potential for iron-chelation applications.

Table 1. Quantitative siderophore chelation efficiency of selected marine bacterial isolates determined using the CAS shuttle assay

Isolate Code	Sampling Site	Siderophore Chelation Efficiency (%)	Production Category
Std. *Bacillus*	Control	43.95	Reference
GSW13	Gorai Marine Water	64.30	High
WS1	Ware Marine Water	58.12	High
EMW1	Elephanta Marine Water	52.48	High
KSW2	Kalbadevi Marine Water	49.47	Moderate
TSW1	Thane Creek	48.26	Moderate

Extraction and Yield of Siderophores

Siderophores were extracted from the top-producing isolates using ethyl acetate. The yield of the extracted secondary metabolites varied between 90 and 120 mg. with isolates GSW13 and WS1 consistently producing higher amounts.

Post-Extraction Siderophore Activity

Post-extraction siderophore activity was evaluated using the CAS agar cup diffusion assay. Decolorization of the blue CAS medium with the formation of light orange halos was observed around wells containing the extracted samples, confirming the presence of iron-chelating activity after extraction. Among the tested isolates, GSW13 exhibited the largest halo, followed by WS1 and EMW1. No color change was observed in solvent control wells, indicating assay specificity. The agreement between qualitative screening, quantitative estimation, and post-extraction activity demonstrates the robustness and reliability of the screening strategy employed in this study.

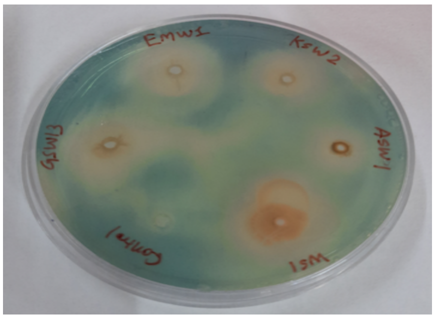

Figure 6. CAS assay for checking the siderophore activity of the extracted secondary metabolite

Molecular Identification of Selected Isolates

Molecular identification by 16S rRNA gene sequencing confirmed that isolate WS1 belonged to *Pseudomonas lundensis*, while isolate GSW13 was identified as *Advenella mimigardefordensis*. Both species are known members of metabolically versatile bacterial groups, supporting their observed capacity for efficient siderophore production.

Conclusion:

The present study demonstrates that marine ecosystems along the Maharashtra coastline harbor diverse siderophore-producing bacterial strains with efficient iron-chelating capability. Systematic isolation and multi-level screening identified a subset of isolates exhibiting high siderophore production, along with pronounced inter-strain variability in chelation efficiency. Among these, isolates WS1 (*Pseudomonas lundensis*) and GSW13 (*Advenella mimigardefordensis*) consistently showed superior iron-chelating activity and retained functionality following solvent-based extraction. Molecular identification provided species-level validation of these efficient producers. Collectively, these findings highlight marine bacteria as a promising source of biologically relevant iron-chelating compounds and provide a foundation for further pharmaceutical-oriented investigations, particularly in the development of siderophore-based drug delivery systems, iron-chelation therapies, and metal-targeted diagnostic or therapeutic agents. In addition, the observed stability and efficiency of the extracted siderophores suggest potential utility in adjunct pharmaceutical formulations and biotechnological applications requiring controlled metal sequestration.

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