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# Isolation and Characterization of Marine Bacteria for Potential Antibacterial Activities

Snigdha Majumder, Astom Mondal and Sankar Narayan Sinha
*Environmental Microbiology Research Laboratory, Department of Botany, University of Kalyani, Kalyani 741235, West Bengal, India*

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**ABSTRACT:**Twelve marine bacterial strain were isolated from seawater from coastal areas of Puri, Odisha, India. The antibacterial activities of these bacteria were investigated. Methanol extracts of marine bacterial fermentation were screened for antibacterial activities through agar well diffusion method. The results indicated that out of 12 strains 7 had antibacterial activities. The active marine bacteria were assigned to the genera Bacillus and Pseudomonas. The TLC autobiographic overlay assay implied that the antibacterial metabolites produced by two strains with wide antibacterial spectrum were different. These marine bacteria were expected to be the potential resources of natural antibiotic products. In order to explore more novel structures, new ways of screening of these compounds should be applied.

**Key words:**Marine bacteria; Antibiotic; Seawater; Bacillus; Pseudomonas.

## I. INTRODUCTION

Compared with terrestrial organisms, the secondary metabolites produced by marine organisms have more novel and unique structures owing to the complex living, circumstance and diversity of species and bioactivities are much stronger. Because of the low content of active compounds in marine animals and plants, as well as limitation of bioresource supply, more and more researchers have been focused on marine microorganisms as sustainable resources.The competition among various microbes for the space and nutrient in marine environment is a powerful selection process which endows marine microorganisms to produce many natural products possessing medical as well as industrial values. Some antimicrobial, antifouling substances have been found among these kinds of bacteria. It is suggested that the primary role of these antibiotic substances could be related to ecological competition. Secondary metabolites of microbial origin are widely used in various fields of human activities, such as medicine, agriculture, pharmaceuticals, food processing, chemical industries and many others. However, few industries have been conducted to study and compare the antibiotic activities of marine bacteria isolated from different origins. With this prospective in mind, the work has been under taken with the following objectives are as isolation of marine bacterium, investigation of antimicrobial properties of these bacterial isolates, characterization and identification of isolated bacterial strains and investigation of the antibiotic compounds produced by different active bacteria were same or not.

## II. MATERIALS AND METHODS

Sampling- Sea water samples were collected in the intertidal zone during low tide from coastal areas of Puri, Odisha, India. Sample water was collected in clean and sterilized glass water bottles and transported to laboratory immediately for bacteriological study.

Isolation of marine bacteria- The sea water sample were serially diluted with sterile 0.85% sodium chloride solution. Aliquots of 0.1ml of diluted samples were spreaded onto 1/10 marine agar plates contain peptone 0.5gm, yeast extract 0.2gm , $FeSO_4$ 0.1gm agar 15gm,sea water 1L and pH was adjust to 7.0-7.6. All plates were incubated at 25℃ for two days. Colonies with different morphology was chosen for further experiment.

TLC autobiography overlay assay- The crude extract of seven marine bacteria (PP1,PP2,PP4,PP6,BP7,BP9 and BP10) with wide antimicrobial spectrum were used in TLC autobiography overlay assay. Each crude extract was dissolved in MeOH and made up to a concentration of 100mg/ml. The solution (2µl) was submitted to TLC analysis on a 3.5x5cm silica gel plate (TLC aluminium sheets 20x20 cm, silica gel 60F 254 Merck Co, USA) using to some types of chemical are (DCM and MeOH) dichloromethane(DCM):EtOAc:Methanol(MeOH) 5:5:1,v/v) as the mobile phase . UV/Vis absorption

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was added for detection at wavelengths of 254nm and 365nm. The developed TLC plates were sterilized by UV lamp for 30 mins before enhanced in the base nutrient agar in a Petri dish (9mm). It was then covered by melting nutrient agar (46℃) containing test bacteria Staphylococcus aureus. After 10 hrs. diffusion process at 8℃ it was then incubated at 37℃ for 24hrs. and the upper agar was sprayed with 5mg/ml of methylthiazoletetrazolium (MTT, Sigma,M5655,USA) to convert to a formazan dye by the test microorganism. Inhibition zones were observed as clear spots against purple background and their Rf values were calculated.

[1].Identification of the bacterial isolates- Bacterial staining, cultural, morphological and biochemical procedures were studied for the identification of bacterial isolate. Isolated bacteria were identified according to methods described in Bergey's Manual of Determinative Bacteriology.

## III. RESULTS AND DISCUSSION

Twelve marine bacteria isolated from sea water samples. The antimicrobial assay showed that 7 strains of these isolates that antimicrobial activity against test bacteria. After taxonomic study it was calculated that the bacteria with antimicrobial activity belonged mainly to the genera Pseudomonas (4 strains) and Bacillus (3 strains). All the seven isolates were then preserved in a slant and broth cultures and were used in studying the colony morphology, microscopical characteristics, gram character, mobility and other biochemical assay which shown in the table-2. On the basis of their 16S rRNA sequence analysis in the twelve strains were identified as Pseudomonas putida and Bacillus subtilis. The separation and identification of bioactive compounds with wide antibacterial spectrum from these marine bacteria is in progress. Crude extracts of two strains PP2 and BP9 with wide antimicrobial spectrum were subjected to autobiographic overlay assay shown in the table-1. Each extracts of different strains showed one or. several inhibition spots under the TLC development system (DCM: EtOAc:MeOH, 5:5:5) and the Rf values of these spots were all found to be different. For extracts of strain PP2 and BP9, the Rf values of inhibition spots were 0.74 and 0.58 respectively.

[2]. In the present study, we have isolated 12 strains from sea water and a large number of bacteria could live on it. These bacterial strains are not actually symbiotic to the host but can instead be considered as associated bacteria (Bultel-Ponce, 1999) with consanguineous relationship with their hosts.[3]. These bacteria, on one hand, could acquire the essential nutrients like sugar, vitamin, fatty acids etc. from their plant or. animal hosts whereas on the other hand they could excrete products like antibiotic, amino acid and toxin propitious for the metabolism as well as development of the hosts or. for the improvement of the chemical defense potential of the hosts.[4,5,6,7]. The present results are quite consistent with the reported previous investigations. The antimicrobial activity of marine bacteria of coastal sea water was reported previously.In our work , the proportion of antimicrobial activity exhibiting bacteria isolated from sea water was near about 58% which were identified as Bacillus and Pseudomonas group. The molecular identification study, in fact, corroborated the identified strains as Bacillus subtilis and Pseudomonas putida. All 7 strains exhibited activity against both gram positive and gram-negative bacteria indicating their broad-spectrum nature.

The results of TLC autobiographic overlay assay demonstrated that different species could produce different antimicrobial metabolites and some process more than one antimicrobial substance.This result implied that some marine bacteria could probably release a number of antibiotic compounds in provide themselves the survival competition superiority.

**Table-1**: Results of antibacterial activity of marine bacteria using agar well diffusion assay.

| Isolated Strain | Antibacterial Activity | | | | | |
|---|---|---|---|---|---|---|
| | Escherichia coli | Vibrio cholerae | Shigella fistula | Shigella dysenteriae | Staphylococcus aureus | Micrococuslateus |
| PP1 | + | + | + | + | + | + |
| PP2 | + | + | + | + | + | + |
| PP3 | - | - | - | - | - | - |
| PP4 | + | + | + | + | + | + |
| PP5 | - | - | - | - | - | - |
| PP6 | + | + | + | + | + | + |

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| | | | | | | |
|---|---|---|---|---|---|---|
| BP7 | + | + | + | + | + | + |
| BP8 | - | - | - | - | - | - |
| BP9 | +++ | +++ | +++ | +++ | +++ | +++ |
| BP10 | + | + | + | + | + | + |
| BP11 | - | - | - | - | - | - |
| BP12 | - | - | - | - | - | - |

'-' no inhibition zone, '+' inhibition zone, '+++' large inhibition zone
PP- Pseudomonas sp. and BP- Bacillus sp.

**Table-2:** Characterization of bacterial isolates.

| Test | Bacterial isolates | | | | | | |
|---|---|---|---|---|---|---|---|
| | PP1 | PP2 | PP4 | PP6 | BP7 | BP9 | BP10 |
| Cell shape | Rod | Rod | Rod | Rod | Rod | Rod | Rod |
| Gram reaction | - | - | - | - | + | + | + |
| Motility | + | + | + | + | + | + | + |
| Growth at 5% | + | + | + | + | + | + | + |
| NaCl | + | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + | + |
| Oxidase | | | | | | | |
| **IMVIC test** | - | - | - | - | - | - | - |
| Indole | - | - | - | - | - | - | - |
| Methyl red | - | - | - | - | + | + | + |
| Voges | + | + | + | + | + | + | + |
| Proskauer | - | - | - | - | - | - | - |
| Citrate | - | - | - | - | - | - | - |
| utilization | - | - | - | - | - | - | - |
| Urease | + | + | + | + | + | + | + |
| $H_2S$ | - | - | - | - | + | + | + |
| production | O/F | O/F | O/F | O/F | F | F | F |
| $NO_3$ | | | | | | | |
| production | | | | | | | |
| Gelatine | + | + | + | + | + | + | + |
| liquefaction | + | + | + | + | + | + | + |
| Starch | + | + | + | + | - | - | - |
| hydrolysis | + | + | + | + | + | + | + |
| Huge | + | + | + | + | + | + | + |
| leifson(O/F) | - | - | - | - | + | + | + |
| reaction | - | - | - | - | + | + | + |
| **Utilization of** | - | - | - | - | + | + | + |
| **carbon** | - | - | - | - | + | + | + |
| **source** | | | | | | | |
| Glucose | | | | | | | |
| Fructose | | | | | | | |
| Xylose | | | | | | | |
| Sucrose | | | | | | | |
| Lactose | | | | | | | |
| Cellobiose | | | | | | | |
| Raffinose | | | | | | | |
| Mannitol | | | | | | | |
| Sorbitol | | | | | | | |

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## IV. CONCLUSION

Marine microorganisms as model systems offer the potential to understand and develop treatments for disease on the basis of normal physiological role of their secondary metabolites and are currently being applied to the development of new drugs. It can be conducted that marine water is a potential source of large number of bacterial strains for sources of new biomolecules that can be exploited to produce antibiotics. In order to find more novel structures new ways of screening of these compounds should be applied.

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**Conflict of interest**

We declare that we have no conflict of interest.

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