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Isolation and Characterization of Marine Bacteria for Potential Antibacterial activities

Astom Mondal[1], Karabi Biswas[2] and Sankar Narayan Sinha[3]
Research Scholar (CSIR-SRF), Department of Botany, University of Kalyani,West Bengal, India[1]
Research Scholar, Department of Botany, University of Kalyani,West Bengal, India[2]
Professor, Department of Botany, University of Kalyani,West Bengal, India[3]

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.
Keywords: 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 main role of these antibiotic substances might be related to ecological competition. Secondary metabolites of microorganisms are used widely in various fields, such as medicine, pharmaceuticals, chemical industries, agriculture, food processing etc. 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 adjusted to 7.0-7.6. All plates were incubated at 25℃ for two days. Colonies with different morphology was chosen for further experiment.

Antibacterial assay: The antibacterial activity of seven marine bacterial suspension was determined using agar well Diffusion method described by Bauer et al (1966) [1] following the standard procedure.

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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 [2].

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 (Table 1). After taxonomic study it was found 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 morphological, physiological and biochemical anlyses, the twelve strains were tentatively identified as *Pseudomonas putida*and *Bacillus subtilis*. Crude extracts of two strains PP2 and BP9 showed greater antimicrobial spectrum (as indicated by larger inhibition zone). In the present study, we have isolated 12 strains from sea water and a large number of bacteria could live on it [3]. These bacterial strains are not actually symbiotic to the host but can instead be considered as associated bacteria [4] with consanguineous relationship with their hosts. 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 [5]. The present results are quite consistent with the reported previous investigations. The antimicrobial activity of marine bacteria of coastal sea water was reported previously [,6,7,8,9]. In our work , the proportion of antimicrobial activity exhibiting bacteria isolated from sea water was near about 58% which were tentatively identified as *Bacillus* and *Pseudomonas* group though molecular identification studyis needed for further confirmation, All 7 strains exhibited activity against both gram positive and gram-negative bacteria indicating their broad-spectrum nature.This result implied that some marine bacteria could probably release a number of antibiotic compounds in provide themselves the survival competition superiority.

TABLE I

Results of antibacterial activity of marine bacteria using agar well diffusion assay ('-' no inhibition zone, '+' inhibition zone, '+++' large inhibition zone, PP- *Pseudomonas* sp. and BP- *Bacillus* sp.).

Isolated Strain	**Antibacterial Activity**					
	Escherichia *coli*	***Vibrio cholerae***	***Shigella flexneri***	***Shigella dysenteriae***	***Staphylococcus aureus***	***Micrococus luteus***
PP1	+	+	+	+	+	+
PP2	+	+	+	+	+	+
PP3	-	-	-	-	-	-
PP4	+	+	+	+	+	+
PP5	-	-	-	-	-	-
PP6	+	+	+	+	+	+
BP7	+	+	+	+	+	+
BP8	-	-	-	-	-	-
BP9	+++	+++	+++	+++	+++	+++
BP10	+	+	+	+	+	+
BP11	-	-	-	-	-	-
BP12	-	-	-	-	-	-

TABLE II

Table-2: Characterization of bacterial isolates (+ presence or positive reaction, - absent or negative reaction, O Oxidation, F Fermentation)

Test	**Bacterial isolates**

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	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	-	-	-	-	-	-	-
VogesProskauer	-	-	-	-	+	+	+
Citrate utilization	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-
NO_3 production	-	-	-	-	-	-	-
Gelatine liquefaction	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	+	+	+
Huge leifson (O/F) reaction	O/F	O/F	O/F	O/F	F	F	F
Utilization of carbon source							
	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Fructose	+	+	+	+	-	-	-
Xylose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Lactose	-	-	-	-	+	+	+
Cellobiose	-	-	-	-	+	+	+
Raffinose	-	-	-	-	+	+	+
Mannitol	-	-	-	-	+	+	+

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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 concluded 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. The separation and identification of bioactive compounds with wide antibacterial spectrum from these marine bacteria is in progress

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BIOGRAPHY

Astom Mondal is currently working as Senior Research Fellow (CSIR-SRF) in the Department of Botany, University of Kalyani, Kalyani, Nadia, West Bengal, India. He has authored over5 research papers in international/national journals. His research interests include environmental research microbiology.

Karabi Biswas is currently working as Senior Research Scholar in the Department of Botany, University of Kalyani, Kalyani, Nadia, West Bengal, India. She has authored over 10 research papers in international/national journals. His research interests include environmental research microbiology.

Dr. Sankar Narayan Sinha is currently working as senior most Professor in the Department of Botany, University of Kalyani, Kalyani, Nadia, West Bengal, India. He has authored over 100 research papers in international/national journals. His research interests include environmental research microbiology.