ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

ASSESSMENT AND DETERMINATION OF HALOPHILIC BACTERIAL DIVERSITY AND ANTIMICROBIAL POTENTIAL FROM MANGROVE ECOSYSTEMS OF BORDI REGION, MAHARASHTRA

Anupama. P. Pathak*[1], Vikas Joshi[1], Supriya Murkute[2], Bhoomi Das[2]

[1]*School of Life Science (DST-FIST & UGC-SAP Sponsored), Swami Ramanand Teerth Marathwada University Nanded, India*

[2]*Department of Microbiology, Nagarjibhai Bhagwanjibhai Mehta Science and Commerce College, Bordi, India*

**Corresponding author: anupama.micro@rediffmail.com*

ABSTRACT

In the tropical and subtropical areas, mangroves are among the most diverse and productive coastal ecosystems. Mangrove forests, which grow at the intersection of terrestrial and marine environments, are home to a wide range of plants, animals, and microbes. The aim of the present study was to explore diversity and distribution of halophilic bacteria and qualitative detection of potential antimicrobial agent from isolate. Hence for study five sampling stations were selected. Rhizospheric sediment of *Avicennia marina* was collected, enriched. Enriched samples were used for isolation on modified marine agar medium. Morphologically distinct 58 isolates were selected. Out of which 6 isolates were used for determination salt tolerance and pH tolerance and antimicrobial activity. All the six isolates were found to have ability to tolerate on 10%- 20% NaCl and pH 9-12. Isolates were named as BS1, BS2, BS3, BS4, BS5 and BS6. Sea water medium was used for production of antimicrobial agent. Antimicobial agent was partially purified by ammonium sulphate precipitation and solvent extraction. Antimicrobial activity of partially purified compound was tested against four human pathogenic strains of bacteria using agar diffusion method. Most of the active strains showed anti-bacterial activity.

Keywords: Mangrove, *Avicennia marina*, Halophile, Halotolerant, Bordi, Antimicrobial compound.

1. INTRODUCTION

The mangrove ecosystem is special environment due to the mixing of saltwater and freshwater during tidal action. A large number of halotolerant and halophilic organisms can prosper in such environmental conditions. Hence mangrove is occupied by salt-tolerant vegetation, flora and fauna and microorganisms that have immense biotechnological potentials. Micro-organisms inhabiting the mangrove ecosystem play a major role in nutrient recycling and in regenerating different forms of the elements used by other life forms [1]. The microbial communities associated with mangrove include all types of microorganisms, like algae, fungi, bacteria and actinomycetes, protozoa etc. which are found in the sediments, water, detritus and which perform certain environmental roles [2]. These are in addition to microorganism also found living in association with other plants and animals. Among the various microorganisms, the bacterial population in mangrove ecosystem is many times greater than that of the others is mainly accountable for most of the nutrient cycle in mangrove ecosystems [3]. It has been constantly observed that marine environments are rich in metabolites with antioxidant properties and therefore they are shows exceptional scope for developing potential drug molecules [4, 5]. Microorganisms surviving in the mangrove forest endure periodic climatic disparity and as a result develop flexibility to huge environmental tension; therefore can be considered a promising source of metabolites. Several halotolerant bacteria inhabiting in the mangrove ecosystem are important source for production of many secondary metabolites which can be used in the formation of drugs.

2. MATERIAL AND METHODS

2.1. Study site

Five Sampling stations of present investigation were identified from Bordi which is 125km away from Mumbai. Mainly tribal community resides, area comes under green belt zone and coastal region is enriched with mangroves specifically *Avicenia marina*. Bordi

having latitude 20.116556 and longitude 72.740013 district Palghar Maharashtra, India, with GPS co-ordinates of 20°6'59.06016'' N and 72°44'24.0468'' E. Five sampling stations chosen were at a 0.5km away from one another.

2.2. Sediment sample collection:

Five sampling area were identified and sediment of rhizosphere of mangrove *Avicenia marina* was collected from these sites namely BS_1, BS_2, BS_3, BS_4, BS_5 and BS_6 collected in pre-sterilized polythene container and transported to laboratory. The temperature was recorded during the sampling and samples were stored at 4°C in freeze until analysis completes.

2.3. Enrichment and Isolation of halophiles:

Sediment samples were enriched by inoculating the composite sediment into pre-sterilized filtered sea water supplemented with carbon and nitrogen source. Inoculated flask was incubated at room temperature for 8 days on a rotary shaking incubator at 120 rpm speed. After enrichment suspension were serially diluted from 10^{-1} to 10^{-10} by using physiological saline (0.85 % NaCl). Last five dilutions were used for isolation. . Modified marine agar medium with 5%, 10%, 15% and 20% NaCl were used and 0.1 ml diluted sample was used for spreading. Plates were incubated at RT for 8 days. Total plate count was noted and morphologically different colonies were selected for further investigation [6-8].

2.4. Differential Staining

An improved technique used for staining of halophilic isolates i.e. Dussault method. In which smear of halophilic bacteria was desalted by using 2% glacial acetic acid and then addition of crystal violet and basic fuchsin as counter stain and observation was recorded [9].

2.5. Biochemical test and sugar fermentation:

Out of 58 isolates six isolates were selected on the basis of their cultural and morphological characteristics for biochemical testing. The six isolates are named as BS_1, BS_2, BS_3, BS_4, BS_5 and BS_6 respectively. The biochemical test for all the six isolates were performed as IMViC, TSI, urease synthesis and sugar fermentation test for glucose, fructose and sucrose.

2.6. Test micro-organisms

The test micro-organisms used in this study, *Escherichia coli* (American Type Culture Collection [ATCC]-8739) *Staphylococcus aureus* (ATCC-6538), *Salmonella abony* (ATCC-6017), and *Pseudomonas aeruginosa* (ATCC-9027) were used. Bacterial suspensions of the specific test species were prepared overnight in Nutrient Broth with constant shaking (120 rpm and 37°C). With sterile saline, the bacterial suspensions were adjusted to the turbidity of 0.5 McFarland standards (about 10^6 CFU/ml).

2.7. Antibacterial activity screening

Primary screening of isolates were performed by crowded plate technique, in which modified agar medium containing desirable percent of salt (10%). Incubate at room temperature (28-30°C). Observe for inhibition zones on plate. Colonies showing inhibition were further used to determine antimicrobial spectrum by giant colony technique against pathogenic organisms. The experiment was performed in triplicates and the results are presented as average with standard deviation [10].

2.8. Active compound extraction:

Antibacterial activity of isolates was investigated further in order to identify potential active compounds. The potent isolates (as established from the initial antibacterial assay) inoculated in 100 ml marine broth and incubated at 37°C for 10 days for extraction of secondary metabolites. The fermentation broth was treated with equal quantities of ethyl acetate, the EtOAc phase was collected, and the process was repeated three times to maximize the extraction. To obtain a broth ethyl acetate extract, the EtOAc phase was evaporated fully under reduced pressure using a rotary evaporator [11, 12].

2.9. Antibacterial activity of active extracts by disk diffusion method

The disc diffusion assay was carried out according to normal protocol, with the test bacteria being spread out on MH agar and sterile Whatman No. 1 filter paper discs loaded with isolate extract in the centre .The plates were then incubated for 48 h at 37°C and the zone of inhibition was observed and recorded [12].

3. RESULTS AND DISCUSSION

3.1. Morphological and biochemical characteristics

Different isolates selection was based on observed growth parameters on media. The isolates with unique growth features were selected for the study and labeled as BS_1, BS_2, BS_3, BS_4, BS_5 and BS_6. The selection of isolates is strictly based on their potential of producing

antimicrobial bioactive compound. Table 1 and 2 shows the growth trait as well as morphological and biochemical characteristics.

3.2. Antibacterial activity from isolates

The antibacterial activity of halophiles isolated from *Avicennia marina* rhizospheric sediment was determined by crowded plate and giant colony technique in which six isolates show inhibition of growth around the colony, forming a distinct zone and their inhibition capacity is measured in mm and depicted in table 3.

Table 1: Biochemical tests of Isolates

Test	BS1	BS2	BS3	BS4	BS5	BS6
Indole	-	-	-	+	-	-
Methyl red	-	-	-	-	-	-
Vogus prauskers	-	-	-	-	-	-
Citrate utilization	-	-	+	+	-	-
TSI	-	-	+A	+AG	-	+AG
Urease activity	+	+	+	+	+	+
Fructose	+	+	-	+	+	-
Dextrose	+	+	+	+	+	+
Sucrose	+	-	-	+	+	+

Note: A-Acid, AG- Acid and Gas, + is for positive and - is for negative result

Table 2: Morphological characters of Isolates

Test	BS1	BS2	BS3	BS4	BS5	BS6
Cell morphology	Rod	Rod	Rod	Rod	Rod	Cocci
Gram nature	Positive	Positive	Negative	Positive	Positive	Positive
Spore formation	+	-	-	-	+	-
Motility	+	+	-	+	+	+
Colony pigmentation	-	Yellow	-	-	Pink	Orange
Optimum NaCl (% w/v)	10	10	17.5	17.5	12.5	10
Salt range (% w/v)	10-15	10-15	10-20	10-20	10-15	10-15
Optimum pH	9	9.5	10	9	9	9.5
pH range	9-12	9-12	9-12	9-12	9-12	9-12

Table 3: Antibacterial activity of the isolates

Name of the isolates	Zone of inhibition (mm)			
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella abony*
BS1	7	6	3	6
BS2	7	9	2	6
BS3	5	5	2	4
BS4	4	12	3	5
BS5	8	7	6	6
BS6	5	2	2	5

3.3. Antibacterial activity

Fermented broth were collected and extracted with the help of ethyl acetate. Extract of each isolate was tested against four pathogenic strains by disc diffusion method. Zone of inhibition was measured in mm which is shown in table 4.

Five isolates were showing effective antibacterial activity against three pathogenic organisms, whereas isolate BS5 showing activity against all four pathogenic strains. From above table it is suggested that isolates showing potent activity as antimicrobial agent against gram positive organism *Staphylococcus aureus* as compared to rest of the three gram negative pathogenic strains.

Table 4: Antibacterial activity of crude extract

Name of the isolates	Zone of inhibition (mm)			
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella abony*
BS1	15	14	-	14
BS2	15	16	-	13
BS3	10	11	-	10
BS4	10	20	-	10
BS5	12	16	13	14
BS6	10	-	-	11

Note: (-) No zone of inhibition

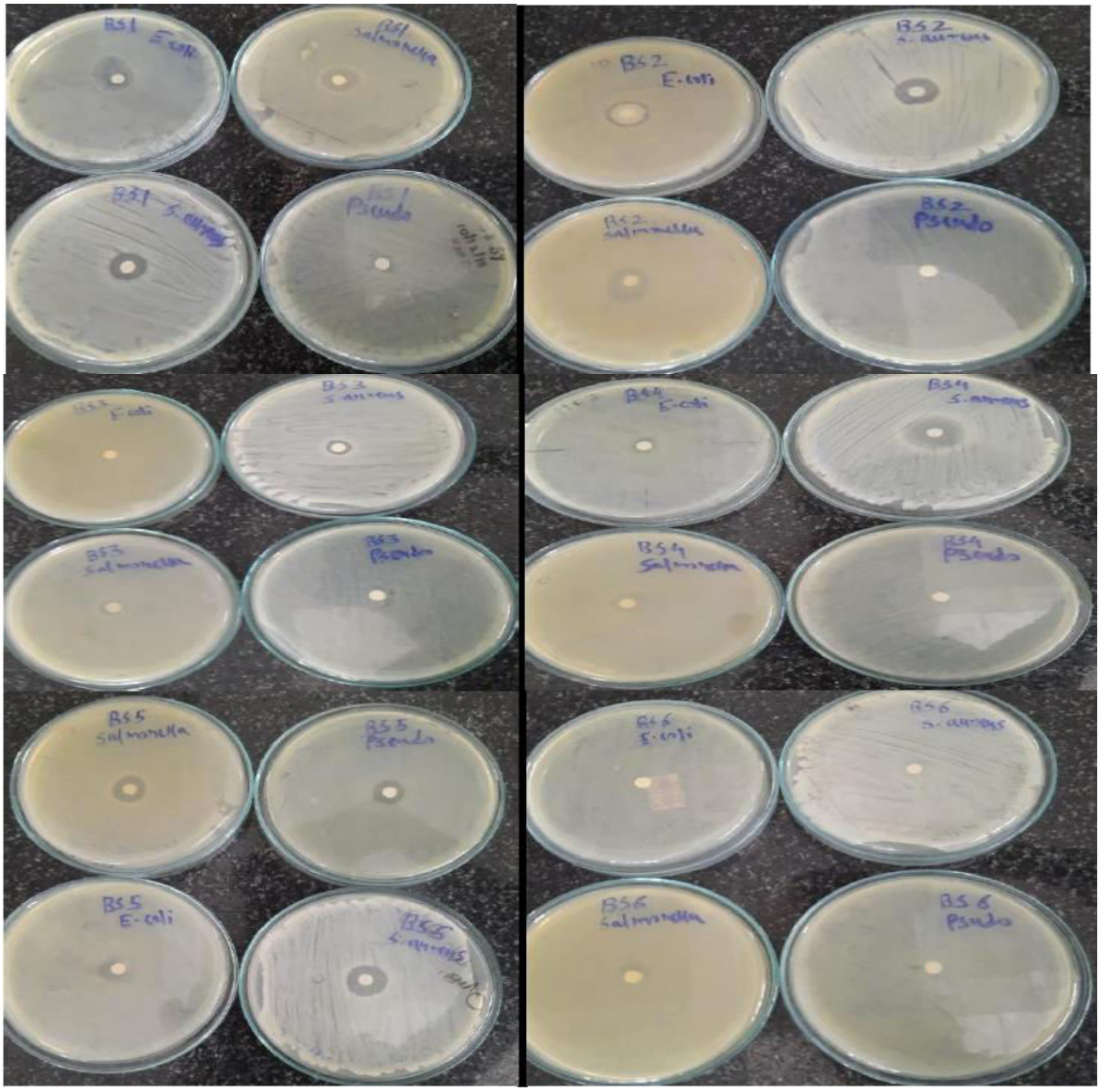

Fig. 1: Antibacterial activity of crude extract

4. DISCUSSION

As the site of study, Bordi is placed in the tribal region, district palghar Maharashtra having coastal area covered with full of diversity. Mangroves spread along the coastal region and covered 20km of area. This coastal region is not yet explored by scientific communities so its fair attempt thus far to explore this area by innervating in halophilic organisms associated with

mangrove sediment. In this study five sites were selected for study and the composite soil sediment associated with mangrove was used as sample for enrichment and isolation of halophilic bacteria. After isolation morphologically distinct 58 isolates were selected. Out of which 6 isolates were used for further investigation. In cell morphology five isolates were rod in shape while one is cocci and others are gram positive in nature while one being negative. All isolates can tolerate salt in range of 10-20% while being optimum at 10, 12 and 17% mostly. Isolates can also tolerate pH ranges from 9-12 and show optimum growth at pH 9 to 10 usually. Three of them also show pigmentation. As dextrose was utilized by all isolates which show that this could be act as a source of carbon. All isolates were producing urease enzyme. These six isolates were then used to detect their antimicrobial activity by initially using crowded plate and giant colony technique which represent isolates have ability to produce antibacterial compound, inhibiting the growth of pathogenic strain. Upon extracting the fermented broth of isolate, the extract was further used for antibacterial activity which shows significant antibacterial activity against four pathogenic strains.

5. CONCLUSION

From above investigation and study it was concluded that halophiles are potent antimicrobial compound producers. This study could provide an insight for further development of novel compound with potential application in biomedicine. Further Characterization of antimicrobial compound of this study by means of Gas chromatography, HPLC,FT-IR,MS/MS need to be performed in order to know the structure,functional group etc. and application in biosciences.

6. ACKNOWLEDGMENT

Authors are grateful to Honorable vice Chancellor of Swami Ramanand Teerth Marathwada University Nanded. Also, Gokhale Education Society's N.B. Mehta Science College Bordi for providing infrastructure facility to carry out the present study.

Compliance with ethical standard

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: This article doesn't contain any studies with human participants performed by any of the authors.

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