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Original Research

Screening for Antibacterial Activity of Marine Bacteria from Seaweeds

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Abstract

A total of 56 bacterial strains were isolated from Seaweeds (*Ulva lactuca, Gracilaria edulis, Chaetomorpha linoides* and *Enteromorpha commpressa*). Isolated bacterial strains were allowed for high throughput screening such as Cross streak method and Disc diffusion method. In the results of cross streak method 23 bacterial isolates exhibited antibacterial activity against 6 human pathogens (*E.coli, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholera, Proteus mirabilis* and *Klebsiella pneumoniae*). These bacterial extracts were allowed for Disc diffusion method. Out of 23 bacterial extracts, only 14 exhibited activity against bacterial pathogens. That strains went for Genus level identification. Two strains namely ENC13 and ENC14 isolated from *E. commpressa* exhibited broad spectrum activity. Both seems to be antibiotic producers. Among these two, ENC13 exhibited high antibacterial activity producing 20mm zone of inhibition. This bacterium exhibiting promising antibacterial activity was identified as *Micrococcus sp*.

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INTRODUCTION

The marine environment is a rich source of biologically active natural products many of which have not been found in terrestrial sources. Natural products are secondary metabolites produced by microorganisms, plants, animals and the biochemical novelty associated with such natural products are higher than that of any other sources. Natural products, the secondary or non primary metabolites produced by living organisms, have been exploited by people for a variety of purpose excluding use food, fragrances, pigments, insecticides and medicines. The marine environment is an exceptional reservoir of bioactive natural products (Ireland *et al*., 1988). Marine natural products encompass a wide variety of chemical classes, including terpenes, shikimates, polyketides, acetogenins, peptides, alkaloids of varying structures and a multitude of compounds of mixed biosynthesis.

The majority of the large conspicuous forms of attached marine plants are the seaweeds. They are in three plant divisions *viz*., Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). The chemical ecology of many of the green algae (Chlorophyta) has been studied extensively, but only cursory pharmacological studies have been done. The novel Sphingosine derivative N-Palmitoyl-z-amino-1,3,4,5-tetra hydroxyl octadecane isolated from the green algae *Ulva faciata* collected off of the West Coast of India has antiviral activity *in vivo* (Garg et al.,1992). Stypoldione was found to be cytotoxic by inhibiting mitotic spindle formation. Stypoldione is used as a pharmacological probe for the study of cell cycles (Jacobs *et al*., 1985). From the red algae *Portieria hornemannii*, a novel cytotoxic penta halogenated monoterpene halomon was isolated. Brain, renal and colon tumour cell line were most sensitive to this compound which has been selected by the NCI Deciesun Network Committee for preclinical drug development. Arachidonic acid, isolated from many algae that convert simple poly unsaturated fatty acids into complex eicosanoids and related oxylipins (Gerwick and Bernart, 1993). In mammalian systems, these arachidonic acid derivatives are important for maintaining normal physiological conditions process known as homeostasis. This is also exhibiting variety of activities including antiviral, antiulcer, antithrombic, antitumour, antilipemic and immunomodulating activity (Carte, 1996).

In this study, the total 56 bacterial symbionts were screened by cross streak and Disc diffusion assay. These two methods have been used as a primary screening tool to assess the antimicrobial potential of the marine bacteria. Such preliminary screening processes are simple, less time consuming and requires only a small quantity of the material. Hence, these protocols are more suitable for screening of marine bacterial extracts, which contains nanogram level of metabolites compare to other macro organisms in the marine environment.

MATERIALS AND METHODS

Collection of Samples

Seaweeds *viz*., *Ulva lactuca, Gracilaria edulis, Chaetomorpha linoides and Enteromorpha commpressa* were collected from the coastal waters of pamban,

Tamilnadu, India. The collected samples were transferred to laboratory in ice box.

Isolation of Bacteria

The Seaweeds were rinsed with sterile seawater prior to analysis to remove loosely attached bacteria (Lemos et al., 1985). For isolation of ectosymbionts, the seaweed surface (approximately 1 cm^2, in three replicates) were swabbed by sterile cotton swab, placed in 2 ml sterile seawater and vortexed. Each solution was diluted 10 times for serial dilution. 0.1 ml aliquots were placed on Zobell marine agar, Actinomycetes agar and B_1 medium (2.5 g peptone,1.5 g yeast extract,1.5 ml glycerol,17 g agar,750 ml filtered seawater,250 ml deionized water).This procedure was repeated in duplicate for each species by swabbing separate surface areas of seaweeds (Wahl *et al*.,1994; Chelossi *et al*., 2004). For isolation of endosymbionts the seaweed sample were homogenized with sterile seawater separately and then the bacteria were isolated in 3 different types of medium as mentioned and incubated at 27°C for 7 days. The individual bacterial strain was isolated by repeated streaking.

Screening for Antibacterial Activity

A total of 56 strains were isolated from seaweeds. Two methods used to determine the antimicrobial activities of isolated bacteria namely (i) Primary screening and (ii) Secondary screening methods.

Primary Screening - Cross Streak Method

The preliminary screening were carried out by following the method of Spragg et al. (1997). The strains were streaked on to TSA plates (Tryptone soya agar + NaCl) and incubated at room temperature for 5 days. Test strains of human pathogens namely *E.coli, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholerae, Proteus mirabilis* and *Klebsiella pneumoniae* were streaked perpendicular to the marine bacterial strains and incubated overnight. Inhibitory activity was indicated by inhibition of growth of pathogenic strains on the agar as compared to a control plate showing healthy growth of the test strains.

Secondary Screening Methods

Preparation of Marine Bacterial Extracts

The active marine strains were inoculated onto 100 ml of Zobell marine broth separately and cultured in a shaker at 290 rpm for 5-7 days at room temperature and then the broth culture was extracted employing liquid-liquid extraction (Gailliot, 1998). Equal volume of ethyl acetate and the broth were stirred for 30 minutes using a magnetic stirrer. The two phase were then separated in a separating funnel and the solvent phase was concentrated by using rotary vacuum evaporator. The concentrated bacterial extracts were used to prepare disc to determine the antibacterial activity.

Disc Diffusion Assay Method

The ethyl acetate extract of active marine bacterial strains were screened against the human pathogens by standard disc diffusion method. The concentrated crude extracts were (approximately 100microgram) impregnated on to sterile Whatman filter paper discs (6mm) and antibacterial activity was assayed following the disc diffusion assay (Acar,1980; Becerro *et al*.,1994). Sterile Muller Hinton agar plates were swabbed with 16 hours old cultures of bacterial pathogens such as *E.coli, P. aeruginosa, S. aureus, V. cholerae, P. mirabilis* and *K. pneumoniae*. The prepared discs were placed on the inoculated plates and the plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured from border of the disc to the edge of the clear zone (Vijayalakshmi *et al*., 2008).

Identification of Marine Strains

The active strains were allowed to identification by using Morphological identification and biochemical identification. Based on the results the strains were identified up to genus level.

RESULTS AND DISCUSSION

Results of preliminary screening for antimicrobial activity against six human pathogens by cross streak method are given in Table 1 and Disc diffusion assay of active bacterial isolates are given in Table 2. Out of the total 56 isolates, 23 were found to be active in preliminary screening. The highest percentage occurrence of antibiotic producer was found in seaweed *E.compressa*. 66.6% of the bacterial isolates exhibited broad spectral activity against all 6 human pathogens. 46.6% activity exhibited by *C. linoides* associated bacteria. 33.3% of activity present in bacteria associated with *U. lactuca* and bacteria associated with *G. edulis* exhibited 17.6% of antibacterial activity (Figure 1).

In the genus level identification of the potential producers, *Pseudomonas* sp., was dominant followed by *Alteromonas* sp., *Alcaligenes* sp., *Micrococcus* sp., *Bacillus* sp., and *Vibrio* sp. (Table 3).

The first attempt to locate antibacterial activity in marine organisms were initiated around the 1950s (Burkholder and Burkholder, 1959). Antibacterial activity of marine bacteria were screened by two methods Cross streak method and Disc diffusion assay. The 56 bacterial strains isolated from seaweeds showed antagonistic activity in cross streak method. Among these 66.6 percent bacteria isolated from *E. compressa* showed activity against at least one bacterial pathogen. This coincide with the study of Lemos et al. (1985) in which 38 strains out of isolated 224 epiphytic bacterial strains from intertidal seaweeds displayed antibacterial activity. Spragg et al., (1997) isolated 51 stains from marine algae *Fucus resiculosus* and in that 13 (25 %) showed activity against Methicillin resistant *Staphylococcus aureus* (MRSA). Jayanth et al., (2002) also reported similar reports in their study on antagonistic marine bacteria against pathogenic bacteria.

13.3% strains isolated from *E. compressa* showed antibacterial activity against minimum four bacterial pathogens. From the *U. lactuca*, 9 strains were isolated out of which 33.3% were found to be active against bacterial pathogens. A total of 17 bacteria isolated from *G. edulis* and 17.6% of which showed antibacterial activity. 15 bacteria were isolated from *C. linoides* and antibacterial activity was exhibited by 46.6% isolates against bacterial pathogens.

Table 1: Cross streak method

Sl.No.	Strain	Bacterial Pathogens					
		Staphylococcus aureus	*Klebsiella pneumoniae*	*Pseudomonas aerugenosa*	*Vibrio cholerae*	*Proteus mirabilis*	*E.coli*
1.	ULL1	-	-	-	-	-	-
2.	ULL2	-	-	-	-	-	-
3.	ULL3	+	-	+	-	-	-
4.	ULL4	-	-	-	-	-	-
5.	ULL5	-	-	-	-	-	-
6.	ULL6	-	-	-	-	-	-
7.	ULL7	+	+	-	+	-	+
8.	ULL8	-	-	-	-	-	-
9.	ULL9	-	+	-	-	+	-
10.	GRE1	+	-	-	+	-	+
11.	GRE2	-	+	-	-	+	-
12.	GRE3	-	-	-	-	-	-
13.	GRE4	-	-	-	-	-	-
14.	GRE5	-	T	-	-	-	-
15.	GRE6	-	-	-	-	-	-
16.	GRE7	-	-	-	-	-	-
17.	GRE8	-	+	-	-	+	+
18.	GRE9	-	-	-	-	-	-
19.	GRE10	-	-	-	-	-	-
20.	GRE11	-	-	-	-	-	-
21.	GRE12	-	-	-	-	-	-
22.	GRE13	-	-	-	-	-	-
23.	GRE14	-	-	-	-	-	-
24.	GRE15	-	-	-	-	-	-
25.	GRE16	-	-	-	-	-	-
26.	GRE17	-	-	-	-	-	-
27.	ENC1	-	+	-	+	-	-
28	ENC2	+	-	+	-	-	+
29.	ENC3	-	-	-	-	-	-
30.	ENC4	-	-	-	-	-	-
31.	ENC5	+	+	+	-	-	-
32.	ENC6	+	-	-	+	-	-
33.	ENC7	-	-	-	-	-	-
34.	ENC8	-	+	-	+	-	-
35.	ENC9	-	-	T	-	-	-
36.	ENC10	-	-	-	-	-	-
37.	ENC11	+	+	-	+	-	+
38.	ENC12	-	-	-	+	-	+
39.	ENC13	+	+	+	+	+	+
40.	ENC14	+	+	+	+	+	+
41.	ENC15	+	+	+			
42.	CHL1	-	-	-	-	-	-
43.	CHL2	-	-	-	T	-	-
44.	CHL3	-	-	+	-	-	-
45.	CHL4	-	-	-	-	+	-
46.	CHL5	-	-	T	-	+	T
47.	CHL6	+	+	-	+	-	-
48.	CHL7	+	-	-	-	-	
49.	CHL8	+	-	-	-	-	-
50.	CHL9	+	-	-	-	-	-
51.	CHL10	-	-	-	-	-	-
52.	CHL11	-	-	-	-	-	-
53.	CHL12	-	-	-	-	-	-
54.	CHL13	-	-	-	-	-	-
55.	CHL14	-	-	-	-	-	-
56.	CHL15	-	-	-	-	-	-

Table 2: Disc diffusion assay method

S.No.	Strain	Bacterial Pathogens					
		Staphylococcus aureus	*Klebsiella pneumoniae*	*Pseudomonas aerugenosa*	*Vibrio cholerae*	*Proteus mirabilis*	*E.coli*
1.	ULL3	12	-	6	-	-	-
2.	ULL7	3	3	-	4	-	3
3.	ULL9	-	8	-	-	2	-
4.	GRE1	2.5	-	-	4.5	-	3
5.	GRE2	-	1.5	2.5	-	-	-
6.	GRE3	-	11	-	-	5	-
7.	ENC1	-	2.5	-	5	-	-
8.	ENC2	8.5	-	1.5	-	-	2
9.	ENC5	1.5	4	T	-	-	-
10.	ENC6	1	-	-	1	-	-
11.	ENC8	-	6.5	-	4	-	-
12.	ENC11	4	3	-	5	-	5.5
13.	ENC12	-	-	-	8	-	2
14.	ENC13	7.5	10	20	17	12	20
15.	ENC14	2	6	15	13	8	5
16.	ENC15	5	-	4	-	-	-
17.	CHL3		T	T	T	-	-
18.	CHL4	5	1.5	T	-	-	-
19.	CHL5	T	T	-	-	-	-
20.	CHL6	2.5	-	2	-	4	-
21.	CHL7	-	T	-	-	-	T
22.	CHL8	-	9	-	1	-	-
23.	CHL9	-	-	-	1.5	T	-

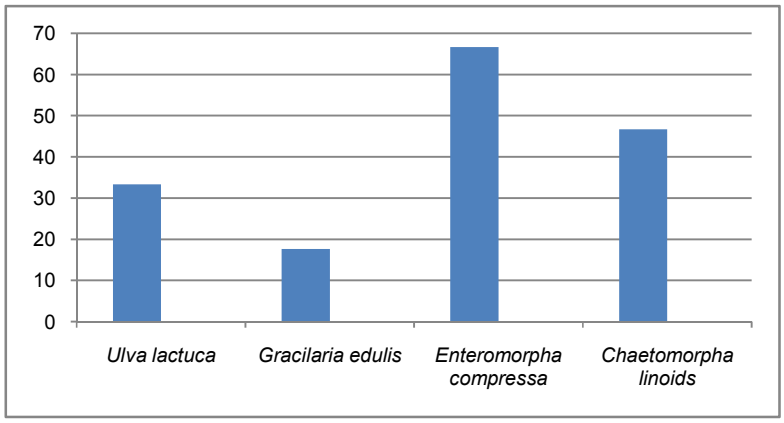

Figure 1: Percentage occurrence of antibiotic producers in Seaweeds

Table 3: Genus level Identification of Potential marine Strains

Sl.No.	Strains	Genus
1.	ULL3	*Alteromonas sp.,*
2.	ULL7	*Alcaligenes sp.,*
3.	ULL9	*Pseudomonas sp.,*
4.	GRE1	*Micrococcus sp.,*
5.	GRE2	*Bacillus sp.,*
6.	GRE3	*Vibrio sp.,*
7.	ENC1	*Vibrio sp.,*
8.	ENC2	*Pseudomonas sp.,*
9.	ENC8	*Bacillus sp.,*
10.	ENC11	*Alteromonas sp.,*
11.	ENC12	*Streptomyces sp.,*
12.	ENC13	*Micrococcus sp.,*
13.	ENC14	*Pseudomonas sp.,*
14.	ENC15	*Alcaligenes sp.,*

CONCLUSION

In the present study, the symbiotic bacterial strains were isolated from Seaweeds (*Ulva lactuca, Gracilaria edulis, Chaetomorpha linoides and Enteromorpha commpressa*). The bacterium ENC13 isolated from *Enteromorpha compressa* showed promising antibacterial activity. Out of the 15 isolates 2 strains ENC13 and ENC14 exhibited broad spectral activity. ENC13 exhibited promising antibacterial activity. The maximum zone of inhibition in antibacterial assay was measured as 20 mm. This strain was identified as *Micrococcus sp*., it may be exploited as a novel drug.

Conflict of Interest

Author declared no conflict of interest.

REFERENCES

Acar, J.F. (1980). The disc susceptibility test. In Antibiotics in laboratory Medicine [V.Lorian Wiliams and Wilkens(eds.)], London, pp.24-25.

Becerro, M.A., Lopez,N.I., Turon,X. and Uniz,M.J. (1994). Antimicrobial activity and surface bacterial film in marine sponges. *Journal of Experimental Marine Biology and Ecology* 179: 195-205.

Burgess, J.G., Jordan, E.M., Bregu, M., Spragg, A.M. and Boyal, K.G. (1999). Microbial antagonism, a neglected avenue of natural products research. *Journal of Biotechnology* 70: 27-32.

Burkholder P.R. and Burkholder, L.M. (1959). Antimicrobial activity in horny corals. *Science* 127: 1174.

Carte, B.K. (1996). Biomedical potential of marine natural products. *Bioscience* 46(4): 271-286.

Chelossi, E., Milanese, M., Milano, A., Pronzato, R. and Riccardi G. (2004). Characterization and antimicrobial activity of epibiotic bacteria from *Petrosia ficifirmis* (Porifera, Demosporgiae). *Journal of Experimental Marine Biology and Ecology* 309: 21-33.

Faulkner, D.J. (2000). Highlight of marine natural products chemistry (1972-1999). *Natural Products Reports* 17: 1-6.

Gailliot, F.P. (1998). Initial extraction and product capture. In methods in Biotechnology Natural Products Isolation [R.J.P. Cannell (ed.), Humana Press, N.J.,U.S.A., 4:53-89.

Garg, H.S., Sharma, M., Bhakuni, D.S., Pramanik, B.N. and Bose, A.K. (1992). An antiviral Sphingosine derivative from the green algae *Ulva fasciata*. *Tetrahedron Letters* 33: 1641-1644.

Gerwick, W.H. and Bernart, M.W. (1993). Eicosanoids and related compounds from marine algae. In Marine Biotechnology: Pharmaceutical and bioactive natural products [Attaway,D.,Zaborsky,O.(eds.)], Plenum Press, New York, 1:101-152.

Ireland, C.M., Roll, D.M., Molinski, T.F., Mckee, T.C., Zabriske, T.M. and Swersey, J.C. (1988). Uniqueness of the marine environment: categories of marine natural products from invertebrates. In Biomedical Importance of Marine Organisms [Fautin,D.G.(ed.)], California Academy of Sciences, San Francisco (CA), pp.41-57.

Jacobs, R.S., Bober, M.A., Pinto, I., Williams, A.B., Jacobson, P.B. and De Carvalho, M.S. (1993). Pharmacological studies of novel marine metabolites. In Marine Biotechnology: Pharmaceutical and Bioactive Natural Products [Attaway, D. and Zaborsky, O.(eds.)], Plenum Press, New York,1:77-99.

James, S.G., Holmstrom, C. and Kjelleberg, S. (1996). Purification and Characterization of a novel antimicrobial protein from a marine bacterium D2. *Applied Environmental Microbiology* 62(8): 2783-2788.

Jayanth, K., Jeyasekaran, G. and Jeya Shakila, R. (2002). Isolation of marine bacteria antagonistic to human pathogens. *Indian Journal of Marine Science* 31(1): 39-44.

Lemos, M.L.,Toranzo, A.E. and Barja, J.L. (1985). Antibiotic activity of epiphytic bacteria isolated from Intertidal seaweeds. *Microbial Ecology* 11: 149-163.

Newman, D.J. and Cragg, G.M. (2004). Marine natural products and related compounds in clinical and advanced preclinical trails. *Journal of Natural Products* 67: 1216-1238.

Proksch, P., Edrada, R.A. and Ebel, R. (2002). Drugs from the sea-current status and microbiological implications. *Applied Microbiology and Biotechnology* 59: 125-134.

Schmidt, E.W. (2005). From chemical structure to environmental biosynthetic pathways: Navigating marine invertebrate-bacteria associations. *Trends in Biotechnology* 23(9): 437-440.

Spragg, M.A., Boyd,K.G., Hubble, M.O. and Burgess, J.G. (1997). Antibiotics from surface-associated marine bacteria, Proceedings of the Fourth under water Science Symposium, Society for under Water Technology, London, ISBN 0906940311, pp.147-157.

Vijayalakshmi, M., Ramasamy, S.S., Murugesh and Murugan, A. (2008). Isolation and Screening of Marine associated bacteria from Tamil Nadu, South east coast of India for potential antibacterial activity. *International Journal Annals of Microbiology* 58(4).

Wahl, M., Jensen, P.R., Fenical, W. (1994). Chemical control of bacteria epibiosis on ascidians. *Marine Ecology Progress and Series* 110: 45-57.