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ANTIMICROBIAL ACTIVITY OF THE SECONDARY METABOLITES EXTRACTED FROM THE ALGAE-ASSOCIATED BACTERIA

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Abstract: Bacteria associated with marine algae are known to have the ability to produce bioactive metabolites. Therefore, an attempt was made to first isolate eight bacterial strains from a marine macro alga *Caulerpa* and then to study the antimicrobial activity of the secondary metabolites produced by these bacterial isolates. Standard paper disc diffusion method was used for the antimicrobial assay. Overall results were highly encouraging as both antibacterial and antifungal activity was exhibited. The results therefore confirmed that the marine algae harbour bacteria having the potential of producing chemicals with antimicrobial activity.

Keywords: Algae, *Caulerpa* sp., bioactive metabolites, antibacterial activity, antifungal activity

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INTRODUCTION

Marine bacteria represent a dramatically different environment for biosynthesis than their terrestrial counterparts and therefore represent a vast untapped resource with potential benefits in many areas such as pharmacology, agro-chemistry and environment. Bacteria have had a profound effect on the development of medical science. Microorganisms are not only the cause of infections but they can also produce organic substances, which can cure infections [1].

Algae are primitive plants with no vascular structure, largely dependent upon water for their sustenance and reproduction. They range in size from microscopic cells the size of bacteria (the blue-green algae) to the massive fronds of the kelps (*Laminaria* spp.)

Associations between algae and bacteria are commonly observed in both freshwater and marine ecosystems, and past culture and microscopy studies have documented a number of bacterial-algal interactions. Algae-associated bacteria are found to be potential in the discovery of bioactive metabolites [2, 3, 4].

The present investigation was carried out to study the antimicrobial potential of the metabolite produced by bacteria isolated from the green alga *Caulerpa* sp.

MATERIALS AND METHODS

Isolation and culture of bacteria from *Caulerpa* sp. (green alga)

Macroalgae *Caulerpa* sp. was collected from the intertidal region of Ratnagiri located in the State of Maharashtra along the west coast of India. Algal sample was transported to the laboratory in a cold sterile condition and used further for the isolation of bacteria.

The samples were washed with sterilized artificial sea water 6-7 times separately to remove any loosely attached micro organisms or any kind of contamination. The samples were then treated with 70% ethanol for 30 seconds. After which they were washed one more time with sterile sea water (ASW). Samples were ground in a mortar pestle with 10 ml sterile sea water. 1 ml out of this was taken out and put in 9ml sterile sea water. 100 µl from this solution was plated on Zobell marine agar (ZMA) plate. These plates were then incubated at 30°C and observed for microbial growth after 24-48 hrs. The bacteria were further purified and maintained on ZMA slants.

Preparation of extracts for antimicrobial screening

The method described by Elyakov et al., was followed in this experiment [5]. Briefly, the eight bacteria isolated from macroalga were fermented in 100ml culture broth contained peptone

(0.25%), yeast extract (0.15%), and glycerol (0.15%) in seawater. The pH was adjusted to between 7.2 and 7.5. The culture flasks were then kept on shaker at 30° C for 48 hrs.

The flasks were removed from the shaker after 48 hrs. The culture broth was extracted by using 30% n-butanol. The contents of the flasks were mixed properly and then separated using a separating funnel. The cell mass and spent medium was discarded and the butanol layer was collected. The butanol extract was concentrated in a Rotary evaporator to evaporate the solvent. The extract was kept in the refrigerator until further use.

Antimicrobial activity of bacterial extracts

The concentrated butanol extracts were tested against test bacterial and fungal cultures. The bacterial strains selected for the assay were *Streptococcus* Sp., *Staphylococcus aureus* and the fungal strains were *Candida albican*s and *Aspergillus fumigates*. Antibacterial and antifungal activities were tested in triplicate by using the standard paper disc diffusion method. The diameter of the inhibition zones was measured in mm after incubation at 37˚C for 24 h. Stock solutions of extracts were prepared by dissolving 5 mg ml^{-1} in methanol. The extracts (100 µg per disc) were applied to sterile paper discs (6 mm in diameter). The solvent was evaporated before they were placed onto agar plates that had been seeded with test bacterial strains. The diameters of the inhibition zones (diameter of inhibition zone minus diameter of disc) were measured in millimeters after incubation at 30°C for 24 hours. Solvent control discs without extracts prepared in the same manner were never observed to inhibit bacterial growth. The standard antibiotics penicillin and streptomycin with 100 µg per disc were used as positive control.

RESULTS AND DISCUSSION

Alga-associated bacteria

Total eight bacterial isolates were isolated from the alga *Caulerpa* sp. The colony characteristics of bacteria are given in table 1.

Table 1 Morphological characteristics of bacteria isolated from alga

Isolate No.	Colony Size (mm)	Elevation	Translucency	Colour
AB1	1	Flat	Translucent	Cream
AB2	1	Umbonate	Translucent	Cream
AB3	1	Raised	Transparent	Cream

AB4	0.5	Raised	Transparent	Cream
AB5	0.5	Raised	Opaque	Yellow
AB6	0.5	Raised	Opaque	yellow
AB7	0.5	Flat	Translucent	Cream
AB8	0.5	Raised	Transparent	Cream

Antimicrobial activity of the extracts

All the eight bacterial isolates were subjected to extraction and the obtained extracts were screened against test bacterial and fungal pathogens. Table 2 highlights the results of antimicrobial activity of bacterial extracts. In this screening bacterial isolate AB6 showed very strong antibacterial and antifungal activity.

Table 2 Antimicrobail activity of bacterial extracts by using disc diffusion method. The activity is given in the form of diameter of inhibition zone.

Bacterial isolate	***Streptococcus* Sp.**	***Staphylococcus aureus***	***Candida albicans***	***Aspergillus fumigates***
AB1	+	++	-	-
AB2	-	++	++	-
AB3	-	-	++	++
AB4	-	-	-	-
AB5	-	-	-	-
AB6	+++	++	+++	+
AB7	++	++	+	+
AB8	+	+	-	-
Streptomycin	+++	+++		
Penicillin			+++	+++

+ Inhibition zone 1 – 2mm, ++ Inhibition zone 2 – 6mm, +++ Inhibition zone > 6mm

It is now well known that the surfaces of marine plants and animals are covered with microbes which play a significant role in the plant development since they evolve allelochemicals capable of protecting the producer from the fierce competition that exists between microorganisms on the surfaces of marine eukaryotes [6]. Such chemically driven interactions are important for the establishment of cross-relationships between microbes and their eukaryotic hosts, in which organisms producing antimicrobial compounds protect the host surface against over colonization in return for a nutrient rich environment [7]. Bioactive compound production in these marine bacteria could be attributed to the competition among them for space nutrition [8]. The production of secondary metabolites inhibits the settlement of other bacteria [9, 10] .

To summarise, the present study clearly indicates that marine algae harbour bacteria which produce antimicrobial substances that can inhibit pathogens. The results are in the agreement with those reported by Kaaria et al., who suggested that marine microbes produce compounds with some degree of bioactivity against other microorganisms or acting against certain physiological states of a diseased body [10]. Authors also showed that different classes of algae (red, brown, green) exhibit different levels of activity. Hence, this creates an avenue for the exploration of the marine algae as a source of antimicrobial compounds that can be used against multidrug resistant microorganisms and come up with novel compounds that can replace the existing drugs once resistance builds up.

CONCLUSION

Macroalgae *Caulerpa* sp. was collected from the intertidal region of Ratnagiri located along the west coast of India. From this algal sample total eight bacterial isolates were obtained. All the bacterial isolates were screened for antimicrobial activity against pathogens. Most of the bacterial isolates showed bioactivity. However AB6 was found to be most potent. Considering its potential, further investigations are ongoing to chemically characterize AB6 bacterial strain and also indentify the bacterium using molecular markers.

REFERENCES

1. Jensen PR and Fenical W: Marine microorganisms and drug discovery: current status and future potential. In: Drugs from the sea (Fusetani N editor), S. Kruger Publishing, Basel, Switzerland (2000) pp. 6-29.

2. Strobel G: Endophytes as sources of bioactive products. Microbes Infect 2003; 5: 535-544.

3. Janakidevi V, YokeshBabu M, Umarani R and Kumaraguru A K: Antagonistic activity of seaweed associated bacteria against human pathogens. Int J Curr Microbiol App Sci, 2013; 2: 140-147.

4. Singh RP, Kumari P and Reddy CRK: Antimicrobial compounds from seaweeds-associated bacteria and fungi. Applied Microbiology and Biotechnology. 2014; 99: 1571-1586.

5. Elyakov GB, Kutznetsova TA and Mikhailov VV: From chemistry of marine natural products to marinetechnologies: Research at Pacific Institute of Bioorganic Chemistry. Mar Tech Soc J. 1996; 30: 21-28.

6. Anahit P, Staffan K and Suhelen E: Development of novel drugs from marine surface associated microorganisms. Mar. Drugs. 2010; 8: 438- 459.

7. Gunatilaka AAL: Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implication of their occurrence. J. Nat. Prod. 2006; 69: 509 - 526.

8. Burgess JG, Jordan EM, Bregu M, Spragg AM and Boyal KG: Microbial antagonism, a neglected avenue of natural products research. J. Biotech. 1999; 70: 27 -32.

9. Holmstrom C, Rittschof D and Kjelleberg S: Inhibition of settlement by larvae of *Balanus amphitrite* and *Ciona intestinalis* by a surface-clonizing marine bacterium. Appl. Environ. Microbiol. 1992; 58: 2111- 2115.

10. Kaaria P, Matiru V, Wakibia J, Bii C and Ndung'u M: Antimicrobial Activity of Marine Algae Associated Endophytes and Epiphytes from the Kenya Coast. Int. J. Curr. Microbiol. App. Sci. 2015; 4(8): 17-22.