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Antibacterial activity of *Chloromonas spp.* isolated from marine ecosystem

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ABSTRACT

Marine Biotechnology is the science in which marine organisms are used in full or partially to make or modify products. The marine environment may contain 80% of world's plant and animal species, and many bioactive compounds have been extracted from various marine animals like lunicates, sponges, soft cords, sea horse, nudibranches, algae, and marine organisms. These compounds were used in pharmacodynamic properties. The water samples were collected from marine ecosystem and the samples were transferred into 100 ml of f/2 medium. After transferring the flask were kept it for incubation at 25°C, at a light intensity of 10 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes and with a light/dark cycle of 14 h / 10 h for one month to get mass growth of microalgae. It was identified as *Chloromonas* spp. One month culture was centrifuged and pellets were collected for extract preparation using acetone, ethanol, chloroform, butanol and petroleum ether. The extracts were used in different concentrations (10μl, 15μl, 20μl and 25μl) to determine the antibacterial activity against Gram positive and Gram negative bacteria viz. *Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella enteriditis* organisms by agar well diffusion method. From that acetone, petroleum ether and ethanol extracts showed greatest antibacterial activity against Gram negative than Gram positive organisms. Particularly acetone extract exhibited higher antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus*.

KEYWORDS

Chloromonas;
Antibacterial;
Microalgae.

INTRODUCTION

Marine biotechnology is the science in which marine organisms are used in full or partially to make or modify products, to improve plants or animals or to develop microorganisms for specific uses. With the help of different molecular and biotechnological techniques, humans have been able to elucidate many biological methods applicable to both aquatic and terrestrial organisms. According to Harvey[10], only 10% of over 25,000 plants have been investigated for biological activity. The marine environment may contain over 80% of world's plant and animal species[29]. In recent years, many bioactive compounds have been extracted from various marine animals like tunicates, sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs, al-

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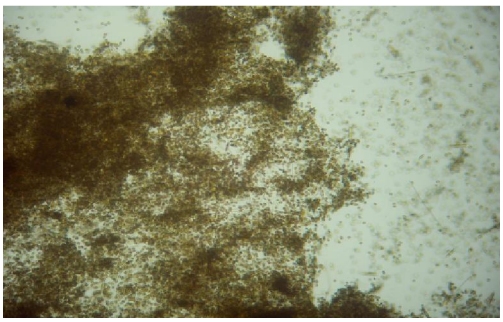
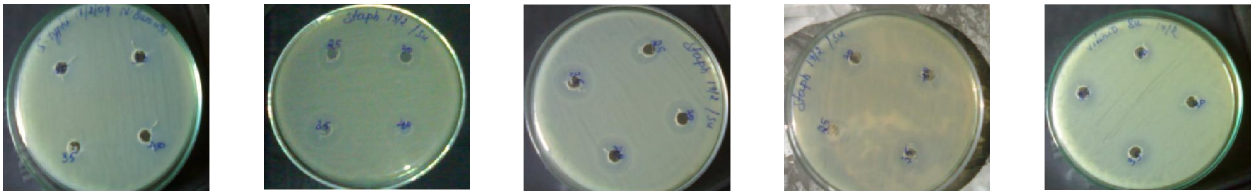


Figure 1 : Microscopic observation of *Chloromonas* spp. (45 x)

(a) (b)

Figure 2 : Mass cultivation of *Chloromonas* spp. with light/ dark cycle of 14 h/10 h for 30 days



(a) (b) (c) (d) (e)

Figure 3 : Inhibition zones obtained by the extracts of *Chloromonas* spp. (a) Ethanol extract against *Salmonella enteritidis*. (b) Acetone extract against *Pseudomonas aeruginosa*. (c) Ethanol extract against *Staphylococcus aureus*. (d) Petrolium ether extract against *E.coli*. (e) Acetone extract against *Bacillus cereus*

gae and marine organisms[7]. The search for new metabolites from marine organisms has resulted in the isolation of more or less 10,000 metabolites[8]. Many of which are endowed with pharmacodynamic properties.

The potential contribution of marine organisms to the discovery of new bioactive molecules is increasingly challenging[13,21,22]. Natural products have been isolated from a wide variety of taxa and tested for various biological activities. Among these taxa, cyanobacteria and algae are regarded as good candidates for drug discovery, with applications in agriculture[3], industry[30] and especially, in pharmaceuticals[12].

Microalgae possess a vast potential in pharmaceuticals, health foods, carotenoids, dyes, fine chemicals, biofuels, etc.,[4]. Some of marine microalgae appear to be potential sources for large scale production of vitamins of commercial interest such as vitamins of B complex group and vitamin E[15]. Microalgae like other plants produce a variety of remarkable compounds collectively referred to as secondary metabolites. They are synthesized by the organisms in cultures at the end of the primary growth phase and into the secondary phase. The relevant substances are diverse in their chemical structure and physiological function. The term bioactive molecule is a slang expression in common use and includes substances which may at low concentration affect life processes beneficial or harmful. More than 50 % of the 100 isolates from marine sources are potentially exploitable bioactive substances. The bioactive substances exhibited antimicrobial activity against a variety of microorganisms.

In spite of being potential producers of a wide spectrum of natural substances of vital human need, microalgae have so far been a rather under explored source in the development of biotechnology[9]. Within status the present study was carried out with the following objectives, isolation and identification of marine microalgae, mass cultivation of marine microalgae with f/2 medium, marine microalgae extracts obtained with organic solvents for antibacterial activity.

MATERIALS AND METHODS

Sample collection

The water samples were collected from Puthiyappa Fishing Harbor, West Cost of India, Calicut. The collected water samples were properly packed and bring to laboratory for further processing.

Isolation and identification

The collected marine samples were transferred into 100 ml of f/2 medium. After transferring the flask were kept it for incubation at 25°C, at a light intensity of 10 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes and with a light/dark cycle of 14 h / 10 h for one week[19]. After one week, the culture flask was subjected to mi-

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croscopic observation. Based on morphology and presence of pigment systems the sample was identified[31].

Mass cultivation of microalgae

Microalgae biomass was produced by cultivating the isolated marine microalgae in f/2 medium. The cultures were maintained at 25°C, at a light intensity of 10 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes and with a light/dark cycle of 14 h / 10 h for 30days. Cells were harvested after 30 days of growth by centrifugation. The pellets were collected and used for further processing. The samples were stored at -4°C[19].

Test organisms

The test organisms used in this work were the following bacterial isolates. All the test bacterial isolates were obtained from fellow mates; they were isolated from clinical samples like pus, urine and sputum samples. The test isolates were subjected to standard antibiogram sensitivity test. The bacterial isolates were incubated into nutrient broth throughout 24 h. The test isolates such as *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella enteritidis*.

Determination of antibacterial activity by agar-well diffusion method

Antibacterial activities of marine microalgae extracts were tested by agar-well diffusion method. Petri dishes with 20 ml for each of nutrient agar, then inoculated with 100 μl of a 24 h broth culture of test bacteria. Indicator microorganisms were spread on agar plates with sterile effusion. Four wells (6 mm) were made and filled with 10, 15, 20 and 25 μl of extract in all the four wells. The inoculated plates were incubated for 24 h at 37°C for bacteria. After incubation, the diameter of the inhibition zone was measured with calipers and the results were recorded in cm[32].

TABLE 1 : Antibacterial activity of green marine microalgae against gram positive and gram negative bacteria

S. No.	Solvents	Concentration	Zone of Inhibition (cm)							
			BC	SA	ML	EC	PA	SE	KP	PV
1	Acetone	10 μl	0.2	-	-	0.5	1.1	-	-	-
		15 μl	0.5	-	-	1.0	1.6	-	0.4	-
		20 μl	0.6	0.4	-	1.3	1.8	0.1	0.7	0.3
		25 μl	0.8	0.5	-	2.0	2.2	0.4	1.2	0.5
2	Ethanol	10 μl	-	0.3	-	0.4	0.3	-	-	-
		15 μl	0.4	0.5	-	0.8	0.8	0.6	0.2	-
		20 μl	0.7	0.7	0.1	1.2	1.1	0.7	0.5	-
		25 μl	0.9	1.0	0.2	1.5	1.4	0.8	0.7	-
3	Chloroform	10 μl	-	-	-	-	-	-	-	-
		15 μl	-	-	-	-	-	-	-	-
		20 μl	0.5	-	-	0.4	0.5	-	0.3	-
		25 μl	0.7	0.4	0.1	0.9	0.8	-	0.5	-
4	Peterolium Ether	10 μl	-	-	-	0.6	-	-	-	-
		15 μl	0.2	0.2	-	0.9	0.6	-	-	-
		20 μl	0.4	0.3	-	1.4	0.7	0.3	0.4	0.2
		25 μl	0.6	0.5	-	1.8	1.1	0.5	0.8	0.6
5	Butanol	10 μl	-	-	-	-	-	-	-	-
		15 μl	-	-	-	-	-	-	-	-
		20 μl	-	0.2	-	0.4	-	-	-	-
		25 μl	-	0.4	-	0.7	0.2	0.1	-	-

BC-*B. cereus*, SA-*S. aureus*, ML-*M. leutus*, EC-*E. coli*, PA-*P. aerogenes*, SE-*S. entritis*, KP-*K. pneumoniae*, PV-*P. vulgaris*, (-)-No zone formation

RESULTS

Out of 10 marine water samples two were showed microalgae growth after one week of incubation. The growth showed samples were examined under high power lens. It showed that green unicellular cylindrical cells. Further, the culture was identified as *Chlorella* spp. based on morphological characteristics and pigmentation by standard algal classification systems described. Microalgae were mass cultivated in f/2 medium. The cultures maintained at 25°C, at a light intensity of 10 μmol μmol m^{-2} s^{-1} provided by cool white fluorescent tubes and with a light/dark cycle of 14 h / 10 h for 30days.

The results obtained from the present study concerning the biological activity if the antibacterial agents produced by green microalgae against three species of Gram positive bacteria and five species of Gram negative bacteria were recorded. It was cleared from the TABLE 1, the diameter of the inhibition zone depends mainly on type of the solvent used and the tested bacterial isolates. Antibacterial activity against Gram positive bacteria was less common than against Gram negative bacteria.

However among the Gram positive bacteria not all the target strains tested were equally susceptible to the antibacterial metabolites produced by microalgae. The two most susceptible organisms were *B.cereus* and

TABLE 2 : Antibiotics sensitivity pattern of testes bacterial isolates

S.No.	Name of Antibiotics	Tested bacterial isolates							
		BC	SA	ML	EC	PA	SE	KP	PV
1	Ciproloxacin (Cf)	S	R	R	I	R	I	R	I
2	Ceftizoxime (Ck)	I	S	S	R	I	R	R	I
3	Gentamycin (G)	R	I	R	R	S	R	S	I
4	Chloramphenicol (C)	S	R	R	I	R	R	R	I
5	Ampicillin (A)	R	S	I	R	R	R	R	R
6	Cefepime (Cpm)	I	R	R	I	S	R	R	R
7	Tetracycline (Tet)	R	R	R	S	R	R	R	R
8	Co-Trimoxazole (Co)	R	I	S	R	R	I	R	S
9	Polymyxin B (Pb)	R	S	I	R	I	R	R	R
10	Streptomycin (S)	S	R	R	S	R	R	S	R

BC-*B. cereus*, SA-*S. aureus*, ML-*M. leutus*, EC-*E. coli*, PA-*P. aerogenes*, SE-*S. entritis*, KP-*K. pneumoniae*, PV-*P. vulgaris*

S.aureus which were inhibited by acetone, ethanol and petroleum ether extracts and showed 0.8 and 0.5, 0.9 and 1.0, 0.6 and 0.5 respectively solvents with concentration of 25 µl. The chloroform and butanol extracts showed very less antibacterial activity against all tested Gram positive bacterial isolates. At the same time *M. luteus* recorded negative antibacterial activity against different solvents extracted of microalgae.

In contrast four Gram negative bacteria (*E. coli, P. aeruginosa, S. aureus, K. pneumoniae*) were showed antibacterial activity against the tested green microalgae. The zone of inhibition was measured 2.0, 2.2, 0.4 and 1.2, respectively at 25 µl of acetone extract. In Gram negative bacteria the acetone extract showed better antibacterial activity than ethanol and petroleum ether extracts. The chloroform extract had moderate activity against *E. coli, P. aeruginosa* but butanol extracts showed very less activity against these bacterial isolates. Negative antibacterial effect was observed towards the *P. vulgaris*. The five tested Gram negative bacteria revealed that the antibacterial effect was greater towards *E. coli, P. aeruginosa* than *S. entrities, K. pnumoniae* and *P. vulgaris*. Concerning the antibacterial effects, the results cleared that acetone and butonal extracts of microalgae gave the greatest biological activity against *E.coli, P.aeruginosa* and *S.aureus*. These results proved that acetone, ethanol and petroleum ether was the best organic solvent for extracting antimicrobial compounds from green microalgae. In addition all the tested pathogens were investigated with the standard antibiotic sensitivity test. The test results were tabulated.

DISCUSSION

The main objective of this work was to screen and evaluate bioactive compounds from marine microalgae and their potential properties of interest. The production of antibacterial activity was considered to be an indicator of the capability, if marine microalgae to synthesis bioactive secondary metabolites.

The percentage of activity observed for the microalgae studied was substantially correlated with data reported in previous study[5]. In our observation go in harmony with those obtained by Volk and Furkert [33], they found that microalgae had high biological activity against *B.subtilis, B.thuringiensis, B.megaterium, E.coli,* and *P.aeruginosa*. In our study result also evidenced with Tuney et al.[24]. Also Ozdemir et al.[14] found that extracts of cyanobacteria and green microalgae obtained by different solvent exhibited antimicrobial activity on both Gram positive and Gram negative organisms.

The higher frequency of activity against Gram positive bacteria also observed in most of the antimicrobial activites from algae reported in literature[2,18]. But in our investigation the Gram negative bacteria showed higher frequency of antibacterial activity. Hence these results were contrast with early report.

All bacteria used for the test were resistant to more than five antibiotics. For example, *S. aureus* was methiciline resistant; Gram negative bacterial strains were resistant to microlids. When the effects of extracts obtained from green microalgae were compound with standard antibiotic test used in this study, it was found that the effective of standard antibiotics was more than that of algal extract on *E.coli*, while the effect of antibacterial agents resulted from algal extracts on *B.subtilis, S.aureus*, and *P.aeruginosa* were higher than those of standard antibiotics. Although the broad spectrum antimicrobial activity of *B.subtilis, P.aeruginosa* and *S.aureus* has been already described b Ballantive et al.[2].

As in this studies reported it was observed that the extracts obtained from various solvents used in this study had antibacterial activities and that those extracts would be much more effective even at low concentration. However, these microalgae had potential source if

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bioactive compounds should be investigated for natural antibiotics.

It is intended that the present work was contribute to understanding and determination of the bioactive material produced by marine microalgae. So, these bioactive compounds will need further studies to identify the chemical structures of these active compounds and to examine their beneficial effect for inhibition of some pathogenic bacteria and fungi. Because, antimicrobial metabolites of algae are of special interest in the development of new harmless green pharmacy.

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