International Journal of Engineering, Science and Mathematics
Vol. 7,Special Issue 4(3), April 2018,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijmra.us, Email: editorijmie@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Antimicrobial potential of marine cyanobacteria collected from the West Bengal coastal region of India against multiresistant microorganisms

Prasenjit Das*

Abstract

This study focuses on some cyanobacteria collected from the Bakkhali, a coastal town of West Bengal, India. The aim of this study was to investigate the antimicrobial potential of some marine cyanobacteria against some standard microorganisms. The sample extracts were tested against four standard multiresistant bacteria and a fungi such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Aspergillus niger*. Cyanobacterial extracts have shown varied levels of antimicrobial properties with *Calothrix sp.* being the most effective as evidenced from MIC and MBC values. These findings are encouraging for further investigation for discovery of new therapeutic agents. Such information may also be of great use in disclosing new sources of already known biologically active compounds.

Keywords:

Cyanobacteria;
Inhibition zone;
MIC;
MBC;
Calothrix sp.

Author correspondence:

Prasenjit Das,
Assistant Professor, Deaprtment of Microbiology,
Vijaygarh Jyotish Ray College, Kolkata- 700032, West Bengal, India
Email: pdmicrobio@gmail.com

1. Introduction

Oceans, seas, coastal backwaters, estuaries, and bays are part of the marine environment. It covers 70.8% of earth's surface and comprises more than 200000 described species of invertebrates and algae [18]. However, it represents only a small percentage of the marine biodiversity [10]. Yet, marine natural products are increasingly receiving attention in the search for new and effective medicinal compounds. Competition for space and nutrients led to the evolution of antimicrobial defence strategies in the aquatic environment. Therefore, aquatic organisms, offer a particularly rich source of potential new drugs [2]. The chemical ecology of marine microbes is vastly unexplored even though microbes produce metabolites that can have significant effects on target organisms [8]. Marine macro-organisms use metabolites from microbial symbionts to deter consumers, subdue prey, and defend their embryos from pathogens [8]. Therefore, a knowledge of the biological activities of marine microorganisms is desirable, not only for the discovery of new therapeutic agents, but because such information may be of value in disclosing new sources of already known biologically active compounds [1].

Marine bioactive compounds, many of which are secondary metabolites, are produced for the purpose of greater survivability or fecundity [11]. Secondary metabolites are adaptive and play a key role in the host's defence against pathogens, parasites, predators, competitors and epibiota [6]. Secondary metabolites as natural products provide greater structural diversity than standard combinatorial chemistry and so they offer major opportunities for finding novel low molecular weight lead structures that are active against a wide range of assay targets [7].

* Assistant Professor, Deaprtment of Microbiology,
Vijaygarh Jyotish Ray College, Kolkata- 700032, West Bengal, India

This study focuses on some cyanobacteria collected from Bakkhali, a coastal town of West Bengal, India. These microorganisms belong to a widespread group of photosynthetic organisms. Cyanobacteria have been considered a rich source of secondary metabolites with potential biotechnological applications in the pharmacological field. Together with production of some potent toxins like hepatotoxic microcystins and nodularins or neurotoxins anatoxins and saxitoxins [4], [16] cyanobacteria produce many substances that are interesting in terms of their antifungal, antibacterial, antiprotozoal, antiviral, anticoagulant and anticancerous activities [17]. These include various classes of secondary metabolites such as polyketides, amides, alkaloids, lactones, peptides, lipopeptides and compounds of mixed biosynthetic origin [9].

The aim of this study was to investigate the antimicrobial potential of some marine cyanobacteria against some standard microorganisms. The sample extracts were tested against four standard multiresistant bacteria and a fungi such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Aspergillus niger*. The extracts of cyanobacteria have shown varied levels of antibacterial as well as antifungal properties. These findings are encouraging for further investigation for discovery of new therapeutic agents. Such information may also be of great use in disclosing new sources of already known biologically active compounds.

2. Research Method

2.1. Microorganisms used:

Escherichia coli, *Pseudomonus aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae* were the test bacteria. All these strains were purchased from National Chemical Laboratory, Pune, India. Bacterial cultures were grown and maintained in Nutrient Agar Media (Himedia, India) at 37°C. *Aspergillus niger* (NCL, Pune, India) was used as the test fungi. It was grown and maintained in Czapek-Dox media (Himedia, India) at 28°C. All the test bacteria, *viz. E. coli*, *P. aeruginosa*, *S. Aureus*, *B. subtilis* and *K. pneumoniae* were found to be resistant against ampicillin and norfloxacin.

2.2. Sample collection:

The cyanobacteria were scraped off rocks and transported to the laboratory in Scott Duran bottles with 50 ml of sterile seawater and washed twice with sterile sea water to remove epizoones, epiphytes, sand, calcareous matter and other adhering detritus matter. Four cyanobacteria belonging to the orders Nostocales and Chroococcales were isolated from the Bakkhali coast and identified by Dr. Gargi Saha Kesh from the Department of Microbiology, Vijaygarh Jyotish Ray College, Kolkata, India.

2.3. Cyanobacterial Biomass Production and Extraction:

The cynobacterial biomass was produced in Erlenmeyer flasks in ASN III Media (NaCl– 25 gm, $MgSO_4.7H_2O$– 3.5 gm, $MgCl_2.6H_2O$– 2 gm, $NaNO_3$– 0.75 gm, $KHPO_4.3H_2O$– 0.75 gm, $CaCl_2.2H_2O$– 0.5 gm, $NaCO_3$– 0.02 gm, Citric acid– 3 mg, Mg-EDTA– 0.5 gm, Ferric Ammonium Citrate– 3 mg, Trace minerals– 1 ml, KCl– 0.5 gm, $KHPO_4.3H_2O$ and $NaCO_3$ were added later in 1lt. distilled water) at 25°C, incubated with shaking at 60 rpm and 12:12 h photoperiod with 2000 lux illumination for 4 weeks.

2.4. Sample preparation:

Cyanobacterial cultures were harvested after 4 weeks by centrifugation at 5000 rpm and extracted with methanol at room temperature. Sample biomass was dried in hot air oven at around 80°C for 36 hours and 5g of dried mass was collected and methanol extract of the dried mass was prepared. The extract was homogenized and centrifuged (Eppendorf A G, Germany) at 9000 rpm for 10 mins at 4°C. Sample supernatants and pellets were separated and vacuum evaporated in vacuum evaporator. The gummy extract was collected in screw-capped vials and stored at 4°C. The aliquots were prepared with methanol and tested for their antimicrobial activity against test microorganisms.

2.5. Assay of antimicrobial property of sample extracts:

Methanol extract of the dried pellets as well as supernatant of each of the samples were tested against the selected microorganisms for antibacterial as well as antifungal property. Methanol was taken as control. 0.1ml of each of the bacterial cultures (~ 6 log CFU/ml) were spread over the Mueller-Hinton agar (Himedia, India) surface in each plate. Five wells of equal diameter were made in each Petri plate over the agar surface by sterilized cork borer at 60° angles apart. The wells were filled with 100 µl of appropriate cyanobacterial extracts. The assay was performed in triplicates of individual Petri dishes. For bacterial cultures, the diameter of the inhibition zone after 24 h of incubation at 37°C was considered to be indicative of bioactivity. The net zone diameter was calculated after subtracting the diameter of the well (5 mm). In case of antifungal assay, Czapek-Dox agar plates inoculated with *A. niger*, were incubated for 72 h at 28°C and inhibition zones were measured as described above.

2.6. Determining the mechanism of antibiosis (bacteriostatic or bactericidal):

For test bacterial cultures, the minimal inhibitory concentration (MIC) was determined by the broth dilution method. Sterilized tubes were filled with 3 ml of the sample extract prepared in Mueller-Hinton broth (Himedia, India). The tubes were incubated at 37°C for 48 h. The experiment was performed in triplicate. In each set, one tube was used as a control (without test microorganism) and another tube was used

as standard (ampicillin and norfloxacin). After incubation, the OD was measured at 610 nm in a spectrophotometer (UV-1800 UV-VIS Spectrophotometer, Shimadzu Scientific Instruments, Japan). MICs were recorded as the lowest concentration inhibiting visible growth. To measure the minimal bactericidal concentrations (MBC), the MIC cultures were plated on fresh Mueller-Hinton agar and incubated for 24 h at 37°C. A reduction of at least 90% of the colonies, compared with the culture of the initial inoculum of the strain, was regarded as evidence of bactericidal activity. When the ratio of MBC/MIC was ≤2 the active fractions were considered to be bactericidal, otherwise they were considered bacteriostatic. If the ratio was ≥16 the fractions were considered to be ineffective.

3. Results and Analysis

The study area, Bakkhali is a coastal town in the district of South 24 Parganas in West Bengal, India. There are many estuaries around this place with the World Famous Sunderban Tiger Reserve nearby. The intertidal and upper subtidal zones of the coast makes it a highly preferred and desirable habitat for a wide range of organisms. However, very little studies have been done so far to explore the bioactive compounds produced by diverse group of marine organisms of this area.

The antimicrobial activity of the cyanobacteria analyzed in this study is shown in table 1. Isolation of bioactive compounds from marine cyanobacteria has been reported in several publications during the last few decades [5], [14]. *Calothrix sp*. was found to be the most active cyanobacteria followed by *Synechococcus sp*. and *Oscillatoria salina*. The methanolic supernatant fraction of the extract from *Calothrix sp*. was found to be inhibitory against all the test bacteria and fungi such as *E. coli*, *P. aeruginosa*, *S. Aureus*, *B. subtilis* and *K. pneumonia* (bacteria) and *A. niger* (fungi). *Synechococcus sp*. was found to be inhibitory against *S. aureus* and *B. subtilis* while *O. salina* showed antibacterial activity against *E. coli* and *K. pneumoniae*. Martins *et al*. [12] and Manilal *et. al.* [11] also reported the antibiotic activity of the marine *Synechococcus sp*. against gram-positive bacteria. However, another cyanobacteria *Chaetoceros sp*. showed no activity against test bacteria, but was found to be effective in inhibiting the growth of the fungi *A. niger* to a significant level. The results of the present study is supporting previous observations reported by several researchers [13], [15].

Marine cyanobacteria	**Mean Inhibition Zone Diameter (cm)**					
	E. coli	***P. aeruginosa***	***S. aureus***	***B. subtilis***	***K. pneumoniae***	***A. niger***
Calothrix sp	1.3 (±0.07)	1.5 (±.05)	1.0 (±0.16)	1.3 (±0.06)	1.3 (±0.18)	1.0 (±0.17)
Synechococcus sp.	0	0	1.3 (±0.011)	1.43 (±0.025)	0	0
Oscillatoria salina	1.0 (±0.17)	0	0	0	1.1 (±0.007)	0
Chaetoceros sp.	0	0	0	0	0	1.5 (± 0.08)

Table 1: Inhibition zone diameter in agar plates after 24 h of incubation of test organisms at 37ºC in presence of respective supernatant fraction of the cyanobacterial extracts. Experiments were performed in triplicate. Results shown with respective SD value.

The MBC/MIC ratios were determined to identify whether the active compounds present in the crude extracts were bactericidal or bacteriostatic compounds. The results of MIC and MBC for the most effective extract, i.e. *Calothrix sp.* are shown in Table 2. Since the MBC/MIC ratios obtained were less than 2, the active components of the crude extract can be considered as bactericidal. The most sensitive microorganism to the extract of the *Calothrix sp*. was *P. aeruginosa*, which showed the highest mean zone of inhibition (1.5 cm) and the lowest MIC (400 µg/ml) and MBC (60 µg/ml) values. Values of the MBC/MIC ratio for other cyanobacterial extracts against test bacterial species showed considerable variations (data not shown). These variations may be due to the chemical nature and quantity of bioactive metabolites present in the crude extract and their mode of action towards different test organisms [3].

Test Bacteria	**Inhibition Zone (cm)**	**Ampicillin (1000 µg)**	**Norfloxacin (1000 µg)**	**MIC (µg/ml)**	**MBC (µg/ml)**	**MBC/MIC**
E. coli	1.3 (±0.07)	-	-	750	80	0.106
P. aeruginosa	1.5 (±.05)	-	-	400	60	0.15
S. aureus	1.0 (±0.16)	-	-	500	100	0.20
B. subtilis	1.3 (±0.06)	-	-	600	90	0.15
K. pneumoniae	1.3 (±0.18)	-	-	600	100	0.17

Table 2: Antimicrobial activities of methanolic extracts of *Calothrix sp.* Experiments were performed in triplicate. Results shown with respective SD values

4. Conclusion

The results presented here indicate that four marine cyanobacterial samples showed antimicrobial properties. Of the 4 species screened, the *Calothrix sp*., was found to be the most potential producer of marine bioactive metabolites capable of inhibiting the growth of all the multiresistant bacteria tested as well as the test fungi. Our results suggest that the search for novel bioactive compounds by screening of different marine flora and fauna could have been an efficient sourcing method. This report shows the antimicrobial activity of the cyanobacterial population from the West Bengal coast of India for the first time. These findings could make ways for exploiting the bioactives present in cyanobacteria in economically as well as ecologically viable manner for better management of various microbial diseases in human and economically important aquatic population also.

References

[1] Bandaranayake, W.M., "Traditional and medicinal uses of mangroves," *Mangroves and Salt Marshes*, vol. 2, pp. 133-148, 1998.

[2] Bansemir, A., Blume M., Schröder S. and Lindequist U., "Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria," *Aquaculture*, vol. 252, pp. 79-84, 2006.

[3] Barbour, E.K., M.A. Sharif, M.A., Sagherian, V.K., Habre, A.N., Talhouk, R.S. and Talhouk, S.N., "Screening of selected indigenous plants of Lebanon for antimicrobial activity," *J. Ethnopharmacol*., vol. 93, pp. 1-7, 2004.

[4] Carmichael, W.W., "Cyanobacteria secondary metabolites – the cyanotoxins," *J. Appl. Bacteriol*, vol. 72, pp. 445–459, 1992.

[5] Falch, B., "What remains of Cyanobacteria?," *Pharm. Unserer. Zeit*., vol. 25, pp. 311-321, 1996.

[6] Harper, M.K., Bugni, T.S., Copp, B.R., James, R.D., Lindsay, B.S., Richardson, A.D., Schnabel, P.C., Tasdemir, D., Vanwagoner, R.M., Verbitski, S.M. and Ireland, C.M., "Introduction to the chemical ecology of marine natural products," in *J.B. McClintock and B.J. Baker (eds.), Marine Chemical Ecology, Marine Biology*, pp. 3-69. CRC Press, Boca Raton, Fla. USA, 2001.

[7] Harvey, A., "Strategies for discovering drugs from previously unexplored natural products," *Drug Discov. Today*, vol. 5, 294-300, 2000.

[8] Hay, M.E., "Marine chemical ecology: what's known and what's next?," *J. Exp. Mar. Biol. Ecol.*, vol. 200, pp. 103-134, 1996.

[9] Kleinkauf, H. and von Dohren, H., "Products of secondary metabolism," in *H.J. Rehm and G. Reed (eds.), Biotechnol*. pp. 308-309. VCH, Weinheim, 1997.

[10] Malakoff, D., "Extinction on the high seas," *Science*, vol. 277, pp. 486-488, 1997.

[11] Manilal A., Sujith S., Selvin J., Kiran G.S., Shakir C. and Lipton A.P., "Antimicrobial potential of marine organisms collected from the southwest coast of India against multiresistant human and shrimp pathogens," *Scientia Marina*, vol. 74(2), pp. 287-296, June 2010.

[12] Martins, R.F., Ramos, M.F., Herfindal, L., Sous, J.A., Skarven, K. and Vasconcelos, V.M., "Antimicrobial and cytotoxic assessment of marine cyanobacteria - *Synechocystis* and *Synechococcus*," *Mar. Drugs*, vol. 6, pp. 1-11, 2008.

[13] Naviner, M., Berge, J.P., Durand, P. and Le Bris, H., "Antibacterial activity of the marine diatom *Skeletonema costatum* against aquacultural pathogens," *Aquaculture*, vol. 174, pp. 15-24, 1999.

[14] Papke, U., Gross, E.M. and Francke, W., "Isolation, Identification and determination of the absolute configuration of fischerellin B- a new algicide from the freshwater cyanobacterium *Fischerella musscicola*," *Tetrahedron Lett.*, vol. 38, pp. 379-382, 1997.

[15] Regunathan, C. and Wesley, S.G., "Control of *vibrio spp.* in shrimp hatcheries using the green algae *Tetraselmis suecica," Asian Fish. Sci*., vol. 17, pp. 147-158, 2004.

[16] Rinehart, K.L., Namikoshi, M. and Choi, B.W., "Structure and biosynthesis of toxins from blue–green algae (cyanobacteria)," *J. Appl. Phycol*, vol. 6, pp. 159–176, 1994.

[17] Vijayakumar, S. and Menakha, M., "Pharmaceutical applications of cyanobacteria- A review," *J. Acute Med*., vol. 5, pp. 15-23, 2015.

[18] Winston, J.E., "The systematist' perspective", in *D.G. Fautin (ed.)," Biomedical Importance of Marine Organisms"*, California Academy of Sciences, San Francisco, pp. 1-6, 1988.