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Discovery

Cyanobacteria, its antimicrobial activity & carbon sequestration capability

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ABSTRACT

Cyanobacteria are known for their biotechnological potential including antimicrobial activities against various pathogens. An attempt was made to study the antimicrobial activity of ether, benzene and distilled water extract of some locally available Cyanobacterial strains like *Anabaena cylindrica.,Oscillatoria princeps., Lyngbya estuarii., Microcystis aeruginosa, Anabaena sp*. against some fungal and bacterial pathogens. The antimicrobial activity of benzene extract of *Microcystis aeruginosa* and *Lyngbya estuarii* showed an effective result against the pneumonia causing organism, *Klebsiella pneumonia.* Benzene extracts of *Oscillatoria princeps* and *Microcystis aeruginosa* was effective against *Enterococcus sp.* Ether extract of *Lyngbya sp.* was most effective against *Enterococcus sp.* Water extract of *Anabaena cylindrica* against *Streptococcus* sp., *Anabaena sp*. isolated from hot spring against *Streptococcus* sp. and *E.coli* and *Oscillatoria* against *Enterococcus sp*. showed maximum inhibitory effect. Benzene, Ether and water extracts of all

experimental cyanobacterial isolates was found to be ineffective against *Aspergillus niger, Penicillium notatum., Fusarium sp., T.mentagophytes,* and *Rhizopus sp.* Among the five organisms of Cyanobacteria studied *Anabaena cylindrica* shows better CO_2 uptake than other four.

Key words: Cyanobacteria, Benzene extract, Ether extract, Antimicrobial activity, carbon sequestration.

Abbreviations: MHA-Mueller-Hinton Agar medium, PDA-Potato Dextrose Agar medium.

1. INTRODUCTION

Cyanobacteria are among the oldest photoautotrophic organisms. They are considered to be one of the potential organisms which can be of use to mankind in various ways. A number of important advances have occurred in cyanobacterial biotechnology in the recent year. Worldwide attention is drawn towards cyanobacteria for their possible use in mariculture, food, feed, fuel, fertilizer, colorant, production of various secondary metabolites including vitamin, toxin, enzymes, pharmaceuticals, pharmacological probes and pollution abatement. Only a few cyanobacterial strains such as *Spirulina* have been well characterized and exploited commercially due to their high protein content. It was observed that some cyanobacteria are having antimicrobial activity. Many filamentous cyanobacteria fix atmospheric nitrogen by means of special cells called heterocysts. Around 5-10% of the cells develop into heterocysts when these cyanobacteria are deprived of both nitrate and ammonia with preferred nitrogen source.

Antimicrobial effect from cyanobacterial aqueous and organic solvent extracts is visualized in bioassays using selected microorganisms as test organisms. The antibacterial activity of cyanobacterial species such as *Oscillatoria sp.*, *Phormidium sp. and Lyngbya majuscula* were tested against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Streptococcus* sp (Vijaya Baskara and Ashok Prabu, 2010). The effect of methanolic extracts of two *Nostoc sp,* five *Scytonema and thirteen Fischerella sp* were studied against fungi like *Candida krusei, Candida albicans, Aspergillus niger, Aspergillus fumigatus* etc. The potential contribution of marine organism to the discovery of new bioactive molecule is increasingly challenging. Natural products have been isolated from a wide variety of taxa and tested for various biological activities.

The antimicrobial activity of *Anabaena flosaquae,Anabaena variabilis* and *Oscillatoria angustissima* against eight Gram +ve and Gram -ve bacteria in addition to two groups of fungi (filamentous fungi and yeast) using agar well diffusion method was studied (Khairy Hanan and. El-Kassas, 2010). Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity. Three cyanobacteria (*Anabaena oryzae. Tolypothrix ceytonica* and *Spirulina platensis)* and two green miocroalgae (*Chlorella pyrenoidosa* and *Scenedesmus quadricauda*) were tested in compliance with the agar well diffusion method for their antibacterial and antifungal agent production on various organism that incite diseases of humans and palnts(*Escherichia coli. Bacillus subtilis, Aspergillus niger, Aspergillus flavus, Candida albicans* etc). It was found that *Spirulina platensis* and *Anabaena oryzae* are the highest antibacterial and antifungal activity towards the tested bacteria and fungi. With increase in population and interest for the discovery of potential source for new drugs in the present work cyanobacteria from local habitats were studied to observe their potential for new bioactive substances that could contribute to reduce the number of harmful bacteria, fungi in our environment.

Global monitoring of atmospheric CO_2 concentration during the last century indicated an increase in carbon dioxide concentration from 295ppm in 1900 to 377ppm in 2004, representing an increase of 27.8% (Thitakamol et al., 2007). On a global basis, it is estimated that more than 25 $GtCO_2$ are emitted annually as a result of burning fossil fuels. The magnitude of the influence of human activities on the biological carbon cycles suggests the need for high managerial levels and the mitigation of emissions of this compound into the atmosphere (IPCC, 2007b).

In this contex, the use of Cyanobacteria having antimicrobial activity can be used for carbon dioxide (CO_2) fixation is considered viable for reducing the emission of pollutants, with this in mind, the objective of the present study includes the study of antimicrobial activity and carbon sequestration potentiality of Cyanobacteria.

2. MATERIALS AND METHODS

2.1. Collection, Identification & Culture

Algal sample were collected from Bindusagar pond, OUAT rice field, Chilka and Otri hotspring. Microscopic observations of algae were made at laboratory under high quality microscope. and the identification of algae taxa was carried out using taxonomic keys after (Prescott et al., 1978) and (Desikachary, 1959).

Five axenic algal strains were selected for screening their antimicrobial activity against some species of bacteria and fungi. Cyanobacteria like *Anabaena cylindrica., Oscillatoria princeps., Lyngbya estuarii., Microcystis aeruginosa, Anabaena sp*. were grown in Allen and Arnon medium, modified freshwater medium and Chu 10 medium respectively. The axenic cultures were grown under controlled laboratory conditions at 27+2°C temperature and 2400 lux light intensity. The organisms like *Anabaena sp*. (Hot spring) was grown at 48^{0}C temperature and 2400 lux light intensity. The organisms were harvested at each 4 days interval. The antimicrobial activity of the cyanobacterial samples were observed on the following test organisms (bacteria and fungus).

2.2. Test organisms

The test organisms used in this piece of work include *Bacillus aereus*, *Enterococcus sp ,Klebsiella pneumonia,Pseudomonas aeruginosa, Streptococcus sp*, *E.coli., Aspergillus niger, Penicillium notatum., Fusarium sp., T.mentagophytes and Aspergillus niger*. The bacterias were grown in MHA (Mueller-Hinton Agar) medium and the fungus were grown in PDA (Potato Dextrose Agar) medium.

2.3. Test for antimicrobial activity

The antimicrobial activity of the cyanobacterial samples were observed using the aqueous extract ,benzene extract and petroleum ether extract of the above mentioned cyanobacterial strains on the above said test organisms (bacteria and fungus). For bacteria disc diffusion method and for fungi pour plate method was used.

2.4. Determination of carbon content and CO_2 fixation rate

The carbon content of the dried algae biomass was determined using the formula:

CO2 fixation rate =0.50P×44/12

Where, P- Biomass productivity; 44- molecular weight of CO_2; 12-molecular weight of carbon

CO_2 fixation rates were calculated from the biomass productivity by using 50% as the carbon content of dried cells (Ugwu et al., 2005)

3. RESULTS AND DISCUSSION

Cyanobacteria shows antimicrobial activity because of the production of secondary metabolites by them .Secondary metabolites influence other organisms in the vicinity .The ability of such compounds to kill bacteria and fungi have been well documented (Browitzka, 1995). The antimicrobial activity of cyanobacteria were evaluated by agar well diffusion method and disc diffusion method for bacteria. The antibacterial activity of brown sea weeds against some human bacterial & fungal pathogens was reported (Manivannan et al., 2011). Pour plate method was used for fungi. The results were summarised in tables 1-7.

The antimicrobial activity of benzene extract of *Anabaena cylindrica., Oscillatoria princeps., Lyngbya estuari., Microcystis aeruginosa, Anabaena sp*. was found to be effective against *Klebsiella pneumonia* (Table 1). Among them *Lyngbya* extract was most effective. E.coli shows positive response towards the extracts of *Oscillatoria princeps* and *Lyngbya estuarii*. Benzene extracts of *Oscillatoria princeps* and *Microcystis aeruginosa was* effective against *Enterococcus sp*. The ether extract of *Lyngbya* was effective against *Enterococcus* (Table 2). The ether extract of *Anabaena* and *Lyngbya* was effective against *Streptococcus*. The antimicrobial activity of hexane , chloroform & ethanol extracts of 6 marine microalgae from north Ceara coast (Northen Brazil) against gram +ve & gram –ve bacteria was reported (Lima-Filho et al. 2002). The antibacterial activity of different organic extracts of *Anabaena variabilis* against *Staphylococcus aureus* MTCC-740, *Escherichia coli* MTCC-739, *Pseudomonas aeruginosa* MTCC-741, *Salmonella typhi* MTCC-733 and *Klebsiella pneumoniae* MTCC-139 was reported (Kaushik et al., 2009).

Water extract of *Anabaena cylindrica* was found to be effective against *Klebsiella pneumonia*, *Streptococcus* sp. *Bacillus cereus*, *Enterococcus* sp. *Lyngbya estuarii* was found to be effective against *Pseudomonas aeruginosa*. Water extracts of *Anabaena sp*.was effective against *E.coli, Pseudomonas aeruginosa* (Table 3). But a significant result was obtained against, *Streptococcus* sp and *E.coli*. using the water extract of *Anabaena sp*.. The efficiency of water extract was found to be more effective than benzene and ether. In this study it was observed that benzene and ether extracts have no effect on *Pseudomonas* but water extract has effect on it.

The inhibition zone of *Anabaena cylindrica* benzene extract against *Klebsiella* was found to be 3 mm where as *Lyngbya* showed the highest inhibition zone i.e. 4mm. *Microcystis* showed inhibition zone of 2 mm against benzene extract of *Klebsiella* (Table 4). Ether extract *Lyngbya* showed highest inhibition zone of 4 mm against *Enterococcus sp* (Table 5).The experimental study revealed that water extract caused bigger clear zones than benzene and ether extracts. The inhibition zone of *Anabaena* sp. showed highest inhibition zone i.e. 5 mm against *Streptococcus sp using* water as the solvent (Table 6).

The growth, productivity and CO_2 sequestration capability of experimental Cyanobacteria were presented (Table 7). Initial biomass (equivalent to 1mg dry wt/25 ml) of algal species were inoculated into the culture medium, the biomass was studied after 20 days. Among the experimental organisms *Anabaena cylindrica* showed better CO_2 sequestration than others.

Table 1

Showing antimicrobial activities of different Cyanobacteria on MHA plates (Benzene), (Disc diffusion method)

Sl.no	Extract	*Anabaena cylindrica*	*Oscillatoria princeps*	*Microcystis aeruginosa*	*Lyngbya estuarii*	*Anabaena sp.*	*Benzene*
1	*Bacillus cereus*	-	-	-	-	-	+
2	*Enterococcus sp*	-	+	+	-	-	-
3	*E.coli*	-	+	-	+	-	++
4	*Klebsiella sp.*	+	+	++	+++	+	+
5	*Streptococcus sp.*	-	-	-	-	-	-
6	*Pseudomonas sp.*	-	-	-	-	-	-

[-: No effect, +: Less effective, ++: Effective, +++: Most Effective]

Table 2

Showing antimicrobial activities of different Cyanobacteria on MHA plates (Ether) (Disc diffusion method)

Sl. no	Extracts of the organism	*Anabaena cylindrica*	*Oscillatoria princeps*	*Microcystis aeruginosa*	*Lyngbya estuarii*	*Anabaena sp.*	ether
1	*Bacillus cereus*	-	-	-	-	-	-
2	*Enterococcus sp*	-	-	-	++	-	+
3	*E.coli*	-	-	-	-	-	-
4	*Klebsiella sp.*	-	-	-	-	-	-
5	*Streptococcus sp.*	-	-	-	+	+	+
6	*Pseudomonas sp.*	-	-	-	-	-	-

[-: No effect, +: Less effective, ++: Effective, +++: Most Effective]

Table 3

Showing antimicrobial activity of different Cyanobacteria on MHA plates (Water) (well diffusion method)

Sl.no.	Extracts of the organism	*Anabaena cylindrica*	*Oscillatoria princeps*	*Microcystis aeruginosa*	*Lyngbya estuarii*	*Anabaena sp.*	Water
1	*Bacillus cereus*	-	+	-	-	-	-
2	*Enterococcus sp*	-	+++	-	-	-	-
3	*E.coli*	-	-	-	-	+++	-
4	*Klebsiella sp.*	+	+	-	-	-	-
5	*Streptococcus sp.*	++	-	-	-	+++	+
6	*Pseudomonas sp.*	-	+	-	+	+	+

[-: No effect, +: Less effective, ++: Effective, +++: Most Effective]

Table 4

Antifungal activity of different Cyanobacterial extracts using benzene as solvent

Sl.no.	Extracts of the organism	*Anabaena cylindrica*	*Oscillatoria princeps*	*Microcystis aeruginosa*	*Lyngbya estuarii*	*Anabaena sp.*	Benzene
1	*Penicillium notatum*	-	-	-	-	-	-
2	*Rhizopus sp.*	-	-	-	-	-	-
3	*Fusarium moniliform*	-	-	-	-	-	-
4	*Aspergillus niser*	-	-	-	-	-	-
5	*T.mentagophytes*	-	-	-	-	-	-

[-: No effect]

Table 5

Antifungal activity of different Cyanobacterial extracts using ether as solvent

Sl.no.	Extracts of the organism	*Anabaena cylindrica*	*Oscillatoria princeps*	*Microcystis aeruginosa*	*Lyngbya estuarii*	*Anabaena sp.*	Ether
1	*Penicillium notatum*	-	-	-	-	-	-
2	*Rhizopus sp.*	-	-	-	-	-	-
3	*Fusarium moniliform*	-	-	-	-	-	-
4	*Aspergillus niser*	-	-	-	-	-	-
5	*T.mentagophytes*	-	-	-	-	-	-

[-: No effect]

Table 6

Antifungal activity of different Cyanobacterial extracts using water as solvent

Sl.no.	Extracts of the organism	*Anabaena cylindrica*	*Oscillatoria princeps*	*Microcystis aeruginosa*	*Lyngbya estuarii*	*Anabaena sp.*	Water
1	*Penicillium notatum*	-	-	-	-	-	-
2	*Rhizopus sp.*	-	-	-	-	-	-
3	*Fusarium moniliform*	-	-	-	-	-	-
4	*Aspergillus niser*	-	-	-	-	-	-
5	*T.mentagophytes*	-	-	-	-	-	-

[-: No effect]

Table 7

Showing the growth, productivity and CO_2 sequestration capability of some Cyanobacteria

Name of Organisms	Mg.dry wt/25 ml	µg.dry wt/25 ml/Day	µg.CO_2 sequestration/25 ml/Day
Anabaena cylindrica	15.9±.09	745±11.12	1365.58±18.23
Oscilatoria princeps	14.6±1.1	730±14.23	1338.09±19.11
Microsystis aeruginosa	13.8±1.3	640±12.11	1173.12±17.88
Lyngbya estuarii	15.5±1.2	725±10.23	1328.92±16.98
Lyngbya sp.	14.9±1.4	695±11.23	1273.93±18.23

4. CONCLUSION

Crude ethanolic extracts from *Anabaena laxa,* inhibited the growth of the following fungi *Asperigillus oryza*, *Penicillium notatum* & *Trichophyton mentagrophyte* (Frankmolle et al. 1992). But the antimicrobial activity of the above said cyanobacterial extract using benzene, ether and water, was found to be ineffective against *Aspergillus niger, Penicillium notatum., Fusarium sp., T.mentagophytes* and *Rhizopus sp.* The ability of blue- green algae to produce antibiotic compounds could be an advantage for their survival in natural environment (Teuscher et al., 1992). A largescale production to obtain sufficient cyanobacterial biomass is the precondition for the isolation and characterization of the antibiotically active compound. The growth, productivity and CO_2 sequestration capability of five experimental Cyanobacteria were better and this observation coincides with the observation of Padhi et al., (2014).

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REFERENCE

1. Browitzka MA. Microalgae as a source of pharmaceuticals and other biologically active compounds, *J. appl. Mphycol*. 1995, 7:3-15.
2. Desikachary TV .Cyanophyta. Indian Council of Agricultural Research, India, 1959.
3. Frankmolle WP, Larsen LK, Caplan FR, Patterson GM, Knubel G, Levine IA, Moore RE. Antifungal cyclic peptides from the terrestrial blue green Anabaena *laxa* .I. Isolation and biological properties. *J.antibiotics*. 1992, 45, 1451-1457.
4. IPCC, Intergovernmental Panel on Climate Change. Mitigation of climate change. http://www.ipcc.ch/., 2007b.
5. Kaushik P, Chauhan A, Chauhan G , Goyal P. Antibacterial Potential and UV-HPLC Analysis of Laboratory-Grown Culture of Anabaena variabilis. *Internet Journal of Food Safety*. 2009, Vol.11: 11-18.
6. Khairy HM, El-Kassas HY. Active substance from some blue green algal species used as antimicrobial agents, *African Journal of Biotechnology*. 2010, Vol. 9(19), 2789-2800.
7. Lima-Filho JV M, Carvalho A FFU, Freitas S M, Melo V MM. Antibacterial activity of extracts of six macroalgae from the north-eastern Brazilian coast, *Brazilian Journal of Microbiology*, 2002, 33:311-313.
8. Manivannan, K, Karthikai Devi G, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar, *Asian Pacific Journal of Tropical Biomedicine,* 2011, 1(2), 114-120
9. Padhi SB, Swain PK, Panda MK, Behera GS, Mohanta R & Panigrahi BN. Efficient CO_2 sequestration by microalgae using photo bioreactor. *International Journal of Biology & Engineering*, 2014, Vol-5, Issue-1. pp1-14.
10. Prescott G, Bamrick J, Cawley E, Jaques W. How to Know the Freshwater Algae, W .C. Brown Co., Dubuque, Iowa, 1978, p. 304.
11. Teuscher E, Lindequist U, Mundt S. Cyanobakterien, Quellen biogener Wirkstoffe. *Pharm. Ztg. Wiss*. 1992, 137: 57–69.
12. Thitakamol B, Veawab A, Aroonwilas A. Environmental impacts of absorption-based CO_2 capture unit for post-combustion treatment of flue gas from coal-fired power plant. *Int. J. Greenhouse Gas Control*, 2007, 1, 318-342.
13. Ugwu CU., Ogbonna JC., Tanaka H., 2005. Light/dark cyclic movement of algal culture (Synechosystis aquatilis) in outdoor inclined tubular photobioreactor equipped with static mixtures for efficient production of biomass. *Biotechnol* Lett. 27:75-78.
14. Vijaya Baskara Sethubathi G , Ashok Prabu V. Antibacterial Activity of Cyanobacterial Species from Adirampattinam Coast, Southeast Coast of Palk Bay. *Current Research Journal of Biological Sciences*, 2010, 2(1) 24-26.