ISSN: 2229- 6905

Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © 2009 PHYCOSPECTRUM - ISSN: 2229 – 6905

Antimicrobial and Antifungal activity of extracts of *Phormidium fragile* Gomont.

N.S. Senthil Kumar , V. Sivasubramanian and S.Mukund

Department of Plant Biology and Plant Biotechnology, R K M Vivekananda College, Chennai 600024, India

Abstract

Cyanobacteria (blue-green algae) are rich sources of structurally novel and biologically active metabolites. Recent studies indicate the presence of some bioactive compounds in the blue green algae which are shown to exhibit anticancer, antimicrobial, antifungal or anti-inflammatory and other pharmacological activities. In the present study, the extracts of Phormidium fragile were assayed for antibiotic activities against the three fungal and three bacterial pathogens.

Introduction

Cyanobacteria are morphologically diverse group of Gram-negative eubacteria. It is able to perform oxygenic photosynthesis and used as important food for other organisms. Moreover, it is widely found in various locations such as pond, soil, rock, bark, sea and fresh water (Carr and Whitton,1982; Castenholz and Waterbury 1989). Cyanobacteria are known to produce a wide range of bioactive compounds. Although, antibacterial, antiviral, algicide, antifungal and cytotoxic activities have been researched in these organisms (Rao, 1994; Issa, 1999; Pushparaj, 1999; Schlegel *et al*., 1999; Schaeffer, *et al* 2000), the properties of the bioactive compounds are still not completely understood (Inderjit and Dakshini, 1994). Cyanobacteria produce many bioactive compounds both intra- and extra cellular to survive in extreme environmental sources (Dvornyk and Nevo,*et al* 2003; Kulik, *et al* 1995; Kreitlow *et al*., 1999; Patterson *et al*., 1994). These bioactive compounds from several cyanobacteria are found to be useful in medicine and agriculture applications (Borowitzka, 1995; Kulik, 1995), such as cryptophycin 1 agent with anticancer (Moore *et al* , 1996; Patterson *et al*., 1991).

The screening of extracts or isolated compounds from different natural sources is a common way to discover biologically active metabolites. Most of the cyanobacterial metabolites are accumulated in the cyanobacterial biomass. Moreover, cyanobacteria excrete various organic compounds into their environment. So, some biologically active compounds were identified among these exo-metabolites e.g. some antibacterial di-terpenoids in *Nostoc commune* (Jaki *et al*., 1999, 2000) or antifungal peptides in *Tolypotrix byssoidea* (Jaki *et al*., 2001) and algicidal phenolic compounds (Volk, *et al* 2005).The antimicrobial substances involved may target various kinds of microorganisms, prokaryotes as well as eukaryotes. The properties of secondary metabolites in nature are not completely understood (Inderjit, 1994). Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenitic importance. Recently, there has been an increasing interest in cyanobacteria as a potential source for new drugs (Schwartz, *et al* 1990). The main objectives of the present investigation were\ to obtain extracts of the filamentous cyanobacterium Phormidium fragile and test them for their antibacterial and antifungal activity.

Materials and Methods

Phormidium fragile was obtained from the culture collection of Vivekananda Institute of Algal Technology (VIAT), Chennai. Improvised CFTRI medium was used for cultivating cyanobacterium (Venkataraman, 1985)

PREPARATION OF CYANOBACTERIAL EXTRACT

0.5g of dried algal material was extracted in 20ml of methanol kept in an orbital shaker for overnight as described by Sivasubramanian *et.al*.2011. The obtained extracts were filtered with Whatman no.1 filter paper and the filtrate was collected. The solvents were removed under reduced pressure at 50°C to yield a concentrated extract (15%).

ISSN: 2229- 6905

AGAR DISC DIFFUSION METHOD:

PREPARATION OF INOCULUM:

Stock cultures of bacteria were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method.

ANTIBACTERIAL ACTIVITY:

Antibacterial activity of cyanobacterial extract was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium was poured in to the Petri plate. After the medium was solidified, the inoculum was spread on the solid plates with sterile swab moistered with the bacterial suspension. The discs were placed in MHA plate with 20 µl of sample. The plates were incubated for 24 hrs, at 37ºC and invhibition zones were measured.

ANTIFUNGAL ACTIVITY:

Preparation of potato Dextrose Broth (PDB):

Antifungal activity of plant extract was determined by antifungal susceptibility test. PDB Broth was prepared and inoculated with the culture and kept in shaker for a day. The potato dextrose agar was weighed as 3.9gms and dissolved in 100ml using distilled water and add 1gm of agar. Then, the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates, these Petri plates were allowed to solidify for twenty minutes. After solidification, the inoculums were spread on the solid plates with sterile swab moistered with the fungal suspension. The discs were placed in PDA plate and add 20 µl of sample. The plates were kept it at Room Temperature. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

Results

Table 1- Antibacterial activity of *Phormidium fragile*

Name of the bacteria	**Zone of inhibition in mm**
	***Phormidium fragile*(Sample)**
Staphylococcus aureus	**12**
Vibrio cholerae	**13**
Salmonella typhi	**13**

Table 2 –Antifungal activity of *Phormidium fragile*

Name of the Fungi	**Zone of inhibition in mm**
	***Phormidium fragile* (Sample)**
Aspergillus Flavus	**12**
Candida Albicans	**13**
Trichoderma viride	**13**

ISSN: 2229- 6905

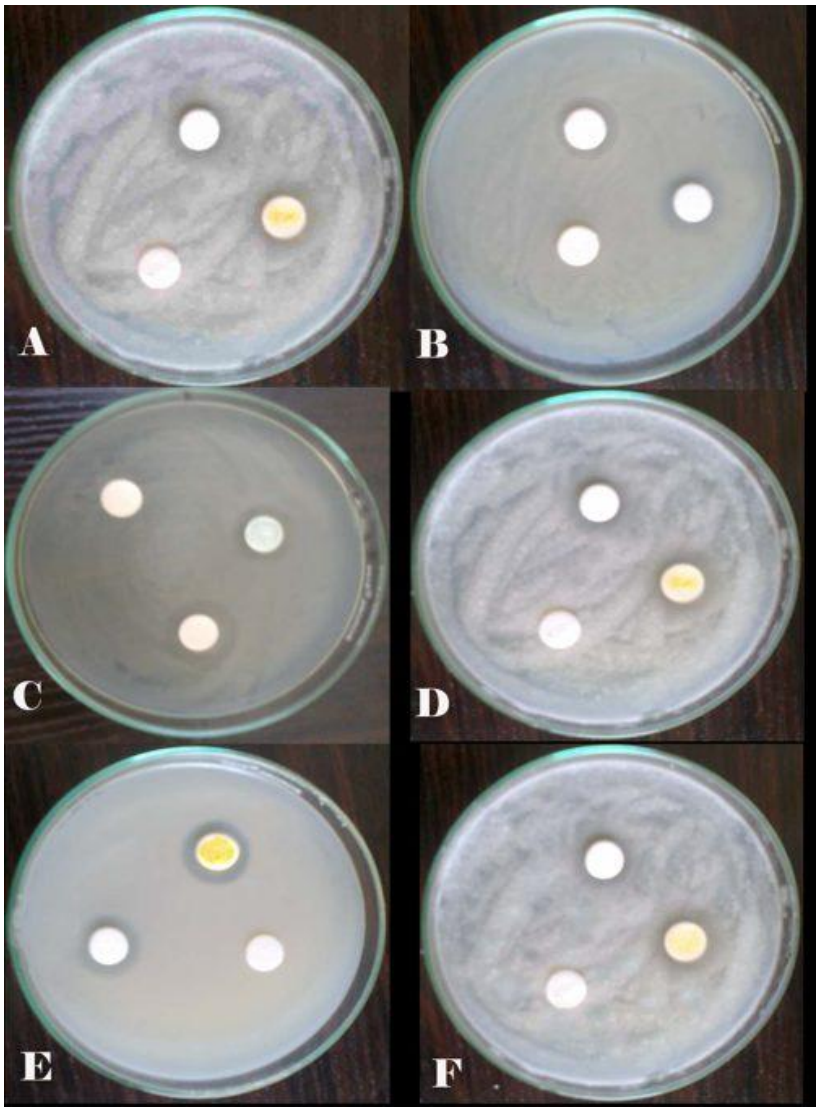

Plate 1 Inhibition zones of methanolic extract of *Phomidium fragile*

A.*Vibrio chlolerae* B *Staphylococcus aureus.* C Salmonella *typhi* D. *Aspergillus flavus* E. *Candida albicans* F *Trichoderma viride*

Results

The antibacterial activity of extracts of *Phormidium fragile* against test bacteria are shown in Table-1. Methanolic extract of *Phormidium fragile* showed a good antibacterial activity against *K.pseudomonas* and *S. aureus*. (Plate 1). The results revealed that all the extracts of *Phormidium fragile* had good activities against the tested bacteria. The maximum inhibition zone was observed against *Staphylococcus aureus* (12mm), *Vibrio cholerae* (13mm) and Salmonella *typhi* (13mm).

The antifungal activity of *Phormidium fragile* against test fungi is shown in Table-2. As the results revealed that *Phormidium fragile* isolates produced a significant inhibition zones and thus antifungal activity. In our study we observed that methanol extracts of the *Phormidium fragile* exhibited potential antifungal activity (Plate 1). The maximum inhibition zone was observed with *Aspergillus flavus* (12mm), *Candida albicans* (13mm) and *Trichoderma viride* (13mm)

Discussion

Nowadays, there is a large availability of clinically useful antibiotics and also a continuous search for new anti-infective agents which remain indispensable. Some of the major antibiotics have indeed considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects. The recent investigations with Cyanobacteria have demonstrated the antimicrobial and antifungal effects of Phormidium sp. (Fish *et al* 1994).These reports are in agreement with our present study, since the methanolic extract of *Phormidium fragile* are much more effective when compared with contemporary antibiotics. The synthesis of highly active toxin is a defence option of Cyanobacteria in these environments against organisms like bacteria, fungi, viruses and eukaryotic micro algae (Mundt *etal* 2001).

It was observed that the methanol was the best solvent for extracting the antibacterial agents from *Phormidium fragile*. An improved knowledge of the composition, analysis, and properties of cyanobacterial strains with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application of blue green algae.

Conclusion

It can be concluded that the methanolic extracts obtained by *Phormidium fragile* can used in this study had substantial antibacterial and antifungal activities and that these extract could be much more effective when compared with contemporary antibiotics and fungicides.

Acknowledgements

We thank the Secretary and Principal, R.K.M. Vivekananda college, Chennai 600 004, India, for providing us with the necessary infrastructure and facilities required for the study.

References

Abdel-Raouf N (2004). Antimicrobial activity of Chlorella vulgaris culture filtrate against some pathogenic microorganisms. *Egypt. J. Biomed.Sci*.**15**: 355-370.

Browitzka, M.A. and L.J. Browitzka, 1988. *Biotechnology*. Cambridge University Press, Cambridge, pp: 456-458.

Borowitzka MA (1988a). Vitamins and fine chemicals from micro-algae.In: Borowitzka, M. A. Borowitzka, L. J. (Eds.), *Micro-algal Biotechnology*. Cambridge University Press, Cambridge,UK. pp.211-217.

Borowitzka MA (1988b). Fats, oils and hydrocarbons. In: Borowitzka, M.A., Borowitzka, L. J. (Eds.), *Micro-algal Biotechnology*. Cambridge University Press, Cambridge, UK. pp.257-287.

Borowitzka MA (1995). Microalgae as sources of pharmaceuticals and other biologically active compounds. *J. Appl. Phycol.* 7: 3-15

Carr NG, Whitton BA (1982). *The biology of cyanobacteria*. Blackwell Scientific Publications Ltd., Oxford.

Castenholz RW, Waterbury JB (1989). *Oxygenic photosynthetic bacteria. Group I. cyanobacteria*, pp. 1710–1727.

De Cano MMS, De Mulé MCZ, De Caire GZ, De Halperin DR (1990).Inhibition of Candida albicans and Staphylococcus aureus by phenolic compounds from the terrestrial cyanobacterium Nostocmuscorum. *J. Appl. Phycol*. **2**: 79-81.

Deskichairy TV (1959). *Cyanophyta*. Indian Council Agricultural research. New Delhi . Academic Press. New York and London. pp. 77-261.
De Mule, M., De Caire, G., De Cano, M., Haaperin, D. (1991). Bioactive compound from *Nostoc muscorum* (Cyanobacterium). *Cytobios*, **66**: 169-172.

Dvornyk V, Nevo E (2003). Genetic polymorphism1. of cyanobacteria under permanent stress: A lessonfrom the "Evolution Canyons". *Res. Microbiol.*, **154**: 79–84.

Geissman, T.A. (1963). Flavonoid compounds, tannins, lignins and related compounds. In: pyrrole pigments, isoprenoid compounds and phenolic plant constituents, vol. 9. *Elsievier*, New York. pp. 265.

Fish, S.A., Codd, G.A. (1994). Bioactive compound production by thermophilic and thermotolerant Cyanobacteria (blue green algae). *World J. Microb. Biotech.*, **10**: 338-347.

Inderjit, Dakshini KMM (1994). Algal allelopathy. *Botanical Rev*., **60**: 182–196.

Inderjit and K.M.M. Dakshini 1994 Effect of cultivation on allelopathic interference success of *Pluchealance olata*. *J Chem Ecol* **20** :1179-1188.

Issa AA (1999). Antibiotic production by the cyanobacteria *Oscillatoria angustissima* and *Calothrix parietina*. Environ. *Toxicol*. *Pharmacol*., **8**:33–37.

Jaki B, Orjala J, Sticher O (1999). A novel extracellular diterpenoid with antibacterial activity from the cyanobacterium Nostoc commune. *J.Nat.Prod*.**62**: 502-503.

Jaki B, Orjala J, Heilmann J, Linden A, Vogler B, Sticher O (2000).Noval extracellular diterpenoids with biological activity from the cyanobacterium Nostoc commune. *J.Nat.Prod*.**63**: 339-343.

Jaki B, Zerba O, Heilmann J, Sticher O (2001). Two novel cyclic peptides with antifungal activity from the cyanobacterium Tolypothrix byssoidea (EAWAG 195). *J. Nat. Prod*.**64**:154-158

Kaushik, P., A. Ghauhan, G. Chauhan and P. Goyal, Antibacterial Potential and UV-HPLC Analysis of Laboratory Grown Culture of *Anabaena variabilis. Inter. J. Food Safety*., **11**: 11-18.

Kailash, N., S.C. Bhardwaj and Y.M. Bahuguna, 2010.from Tapoban Geothermal field, Uttarakhand Himalaya for the Production of Antibacterial Compounds. *Asian J. Exp. Biol. Sci*., **1**: 787-791.

Kulik MM (1995). The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. *Eur. J. Plant Path*., **101**(6): 585-599.

Kreitlow S, Mundt S, Lindequist U (1999). Cyanobacteria- A potential source of new biologically active substances. *J. Biotech.*, **70**: 61-63.

Mundt, S., Kreitlow, S., Nowonty, A., Effrmert, U. 2001. Biological and pharmacological investigation of selected cyanobacteria. *Int. J. Hyg. Environ. Health.*, **203**: 327-334.

Moore RE (1996). Cyclic peptides and depsipeptides from cyanobacteria: a review. J. Ind. Microbiol., 16: 134–143.

Noaman N.H., Fattah, A., Khaleafa, M., Zaky, S.H. (2004). Factors affecting antimicrobial activity of *Synechococcus leopoliensis*. *Microbial Reasearch*, **159**: 395- 402.

Schaeffer DJ (2000). Anti-HIV activity of extracts and compounds from algae and cyanobacteria. *Ecotoxicol. Environ. Saf*., **45**:208–227.

Schwartz RE, Hirsch CF, Sesin DF (1990). Pharmaceuticals from cultured algae. *J. Ind. Microbiol*., **5**: 113-124.

Rao CSVR (1994). Antimicrobial activity of cyanobacteria. I. *J. Mar. Sci*., **23**: 55–56.

Patterson GML, Baldwin CL, Bolis CM, Caplan FR, Karuso H, Larsen LK, Levine IA, Moore RE, Nelson CS, Tschappat KD, Tuang GD, Furusawa E, Furusawa S, Norton TR, Raybourne RB (1991). Antineoplastic activity of cultured blue-green algae (Cyanophyta). *J. Phycol*., **27**: 530–536.

Pushparaj B (1999) Toxicological analysis of the marine cyanobacterium *Nodularia harveyana*. *J. Appl. Phycol*., **10**: 527–530.

Pedersen M, Da Silva EJ (1973). Simple brominated phenols in the blue-green alga Calothrix brevissima *West. Planta*. **115**: 83-96

Ramamurthy VD (1970). Antibacterial activity of the marine blue-green alga *Trichodesmium erythraeum* in the gastro-intestinal tract of the sea gull *Larus brumicephalus*. *Mar.Biol.* **6**:74-76.

Rainer, B.V., 2006. Antialgal activity of several cyanobacterial exometabolites. *J. Appl. Phycol*., **18**: 145-151.

Safonova E, Reisser W (2005). Growth promoting and inhibiting effects of extracellular substances of soil microalgae and cyanobacteria on Escherichia coli and Micrococcus luteus. *Phycol. Res*.**53**:189-193.

Saxena S, Pandey AK (2001). Microbial metabolites as eco-friendly agrochemicals for the next millennium. *Appl. Microbiol. Biotechnol.,* **55**: 395–403.

Schlegel I, Doan NT, de Chazal N, Smith GD (1999). Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *J. Appl. Phycol*., **10**: 471–479.

R. Uma, V. Sivasubramanian and S. Niranjali Devaraj.2011. Evaluation of in vitro antioxidant activities and antiproliferative activity of green microalgae, *Desmococcus olivaceous* and *Chlorococcum humicola*. *J. Algal Biomass utln*., **2** (3): 82– 93.

Smith GM (1950). *The freshwater algae of the United States.* Mc. Graw-Hill, New York, USA.

.