# Screening of cyanobacterial strains for antibacterial activity

**T.Malathi, M.Ramesh Babu, T.Mounika, D.Snehalatha** and **B.Digamber Rao***

Algal Biotechnology Laboratory, Department of Botany, Kakatiya University, Warangal-506 009, AP, India.
E mail: *bdr_kuc@yahoo.co.in*

**Abstract**

Three Cyanobacterial strains such as, *Tolypothrix tenuis, Anabaena variabilis* and *Cylindrospermum* sp. were isolated from the soil samples collected from paddy fields of Telangana State in sterilized nitrogen free BG-11 medium. Antimicrobial activities of these three strains were studied. Chloroform, Methanol and Water extracts from the biomass of selected Cyanobacteria were isolated and screened against two strains of bacteria (*Bacillus subtilis* (ATCC-11774) and *Pseudomonas auruginosa (*ATCC-15442). The growth of the bacterial strains tested were inhibited by the culture extracts prepared by using different solvents Chloroform, Methanol and Water in which Chloroform extracts of Cyanobacteria have shown maximum inhibition zones under investigation.

**Keywords:** *Cyanobacteria (Tolypothrix tenuis, Anabaena variabilis* and *Cylindrospermum* sp.), *Solvent extracts, Bacterial strains, Antibacterial activity, Zone of inhibition.*

## Introduction

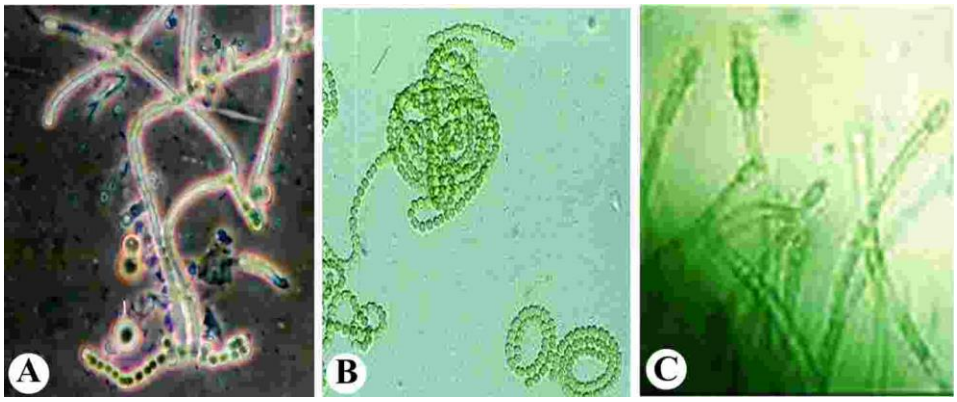
Cyanobacteria are rich sources of structurally novel and biologically active metabolites. Recent investigation on biologically active secondary metabolites from Cyanobacteria led to the identification of wide range of compounds processing antimicrobial, antiviral, antineoplastic and toxic properties (Falch *et al*., 1995; Moore, 1996 and Namikoshi & Rinehart, 1996). Cyanobacteria are characterized by their capacity to perform biological nitrogen fixation and oxygenic photosynthesis. Cyanobacteria are very resistant to extreme environmental conditions and even they tolerate to high temperature upto 50ºC. They are assuming increasing importance in frontier areas of biotechnology. The typical anabiosis and rapid restoration of activity under favorable condition are characteristics of them (Pankratova, 1987).The antimicrobial substances involved may target kinds of microorganisms, prokaryotes as well as eukaryotes. The properties of secondary metabolites in nature are not completely understood (Metting and Pyne, 1986, Inderjit and Daskshini, 1994). Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetic importance. Antimicrobial effects of Cyanobacterial aqueous and organic solvent extracts are visualized in bioassays using selected microorganisms as test organisms (Frankmoll *et.al*, 1992). Bacterial bioassay comprise different test bacteria, *Bacillus subtilis* and *Pseudomonas auruginosa* that are commonly used to find out antibiotic residues in food. In the present study the antibacterial activity of cell extracts of Cyanobacteria (*Tolypothrix tenuis, Anabaena variabilis* and *Cylindrospermum* sp.) in vitro against both Gram-positive and Gram-negative pathogenic bacteria (*Bacillus subtilis* and *Pseudomonas auruginosa*) was investigated.

## Materials and Methods

### *Isolation and culture conditions*

Soil samples were collected from different agro-climatic region of paddy fields of Telangana State. Soil samples in laboratory were cultured in BG-11 medium with or without nitrogen source, after colonization, Cyanobacteria was transferred to fresh medium. Unialgal cultures were prepared using sub culture method. Each isolated was cultured in a 500 ml flask containing 150 ml of BG-11 medium without shaking, for 30 days. The incubation temperature was 25±2ºC and illumination at 4000 lux with a white continuous light and a regime of 16hr light / 8hr dark. Three strains, *Tolypothrix tenuis, Anabaena variabilis* and *Cylindrospermum* sp. were isolated by standard plating and streaking techniques **(Fig.1)**.

**Figure 1: Photographs of Cyanobacterial species**




*A. Tolypothrix tenuis* **B.** *Anabaena variabilis* **C.** *Cylindrospermum* sp.

***Identification of cyanobacteria***

Identification of the Cyanobacteria was done by using morphological variation studies and taxonomical approaches mentioned in the published literature of Desikachary (1959), Anand (1989) and Santra (1993).

***Preparation of cell extracts***

The Cyanobacteria cultures were harvested after one-month of growth by centrifugation at 5000 rpm for 15 minutes. In each case, the algal pellet were collected, weighted and used for extraction of antibacterial agents. One gram of dried powder of each three algal pellets were extracted in 10 ml, either with chloroform, methanol and water to get extract compounds with increasing polarity by shaking overnight for complete extraction. The extract were filtered and the filtrate concentrated under reduced pressure at 37-40ºC and were stored at -20ºc for further studies. The concentration was adjusted to 1mg / ml by using the same solvent used for extraction was assayed for antibacterial activity.

***Antibacterial screening assay***

Antibacterial activities of the each water, methanol and chloroform extracts of to *Tolypothrix tenuis, Anabaena variabilis, Cylindrospermum* sp. were determined by the paper disk (6 mm) diffusion method. Two strains of bacteria were used as test organisms Gram-positive bacteria, *Bacillus subtilis* (ATCC-11774) and Gram-negative bacteria, *Pseudomonas auruginosa (*ATCC-15442). The bacteria used were collected from agar slants which were less than 30 days old. Loop full samples taken from the slants were grown in sterile 50ml broth culture which had been autoclaved at 121ºC under a pressure of 15 lb $in^{-2}$ for 15 minutes and kept to grow for 16 hr at 37ºC. The nutrient broth for bacteria growth was composed of Nacl (5g), peptone (5g), yeast extract (2g), and distilled water 1 litre. Agar plates for the paper disks diffusion test against bacteria were prepared by adding Agar (15g), Nacl (5g), peptone (5g), yeast extract (2g), and distilled water to make it one litre. The media were sterilized as for the nutrient broth media, and 20 ml of media were poured into sterile petridishes. Petri dishes were allowed to cool and solidification, then inoculated with 100 µl of Na 24 hr broth culture of test bacteria. Indicator microorganisms were spread on Nutrient agar plates with a lawn of cultures. Three sterilized blank paper discs (6.0 mm) impregnated with each 50 µl of extracts, and dried under laminar air flow and placed on the inoculated agar plate medium. Plates were incubated at 37ºC for 24 hours. After incubation, each plate was examined and the diameter of the zones with complete inhibition of growth, including the diameter of the paper disc, were measured and expressed in mm. For sensitivity control of agar plates, standard antibiotic Gentamycin (10µg) discs were used as control for reference purposes. All tests were made in triplicate under sterile conditions.

The following formula was used for comparison of the antimicrobial activity of the sample with that of the standard (antimicrobial index):

$$\frac{\text{Inhibition zone of sample}}{\text{Inihibition zone of the standard}} \times 100$$

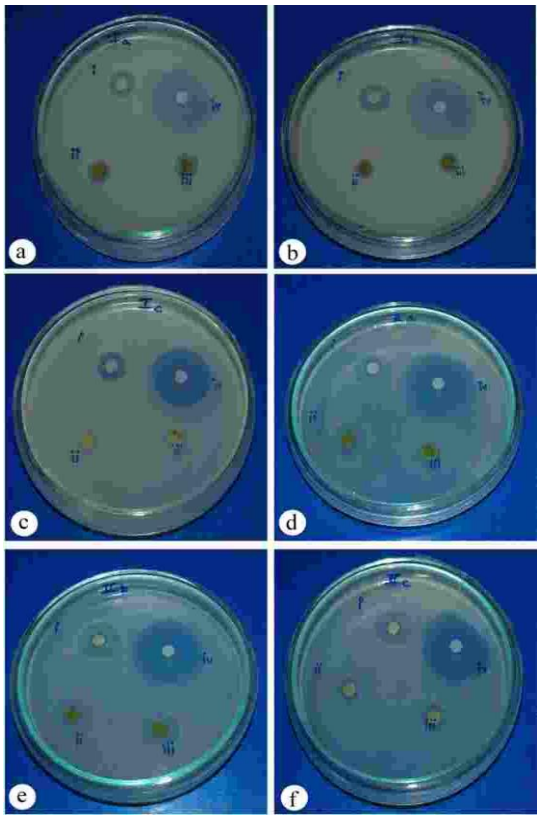
***Statistical analysis***

The results of the data were statistically analysed by using standard error. The values are mean ± standard error **(***S.E***)** of three measurements (N=3).

**Results**

The results obtained from the present study concerning the biological activity of the antibacterial agents producing some selected Cyanobacteria against Gram-positive bacteria, *Bacillus subtilis* (ATCC-11774), and Gram-negative bacteria, *Pseudomonas auruginosa* (ATCC-15442), are presented in **Table 1**. It is clear that the diameter of the inhibition zone depends mainly on type of the algal species, type of the solvent used and the tested bacterial organisms. In the present study, extracts of *Tolypothrix tenuis, Anabaena variabilis* and C*ylindrospermum* sp. with three different solvents namely methanol, chloroform and aqueous extracts respectively. The antibacterial potential of the cyanobacterial strains with different extracts is shown in **Table 1.** The results indicate that the maximum sensitivity measured in terms of zone of inhibition against gram negative bacteria *Pseudomonas auruginosa* was noticed in the methanol extract of C*ylindrospermum* sp. (11.7 mm) followed by chloroform extract of *Anabaena variabilis* (11.6 mm) and methanol extract of *Anabaena variabilis* (11.3 mm). A moderate inhibitory activity was shown by chloroform extracts of *Tolypothrix tenuis* (10.6 mm) and C*ylindrospermum* sp. (10.3mm) against *Pseudomonas auruginosa* and the same moderate activity was also observed in chloroform extracts of *Tolypothrix tenuis* (10.0 mm) against *Bacillus subtilis.* The remaining extracts of the three cyanobacterial cultures showed very less inhibitory activity against the respective bacterial species. Thus, the above results proved that methanol was the best solvent for extracting the antibacterial agents from C*ylindrospermum* sp. and chloroform was the best solvent for extracting antibacterial agents from *Anabaena variabilis* **(Fig.2)**. The antimicrobial activity of the test microorganisms against standard antibiotic was found that the effect of the standard antibiotic Gentamycin (10µg/ml) was more than that of Cyanobacterial extracts on the *Bacillus subtilis* and *Pseudomonas auruginosa* respectively.

**Figure 2: Antibacterial activity (zone of inhibition) from crude extracts of *Tolypothrix tenuis, Anabaena variabilis,* C*ylindrospermum* sp. against *Bacillus subtilis* and *Pseudomonas auruginosa*.**




**A).** Petri dishes **a, b, c** shows *Tolypothrix tenuis, Anabaena variabilis,* C*ylindrospermum* sp. antibacterial activity against *Bacillus subtilis* (ATCC-11774).

**B).** Petri dishes **d, e, f** shows *Tolypothrix tenuis, Anabaena variabilis,* C*ylindrospermum* sp. antibacterial activity against *Pseudomonas auruginosa* (ATCC-15442).

i). Chloroform extract
ii). Methanol extract
iii). Water extract
iv). Control (Gentamycin 10µg).

**Table 1:** Antibacterial activity of three Cyanobacterial crude extracts against to test microorganisms, *Bacillus subtilis* (ATCC-11774) and *Pseudomonas auruginosa (ATCC-15442)*

| Cyanobacteria | Nature of Crude extract | Zone of Inhibition (mm) Antibacterial activity against | |
|---|---|---|---|
| | | *Bacillus subtilis (ATCC-11774)* | *Pseudomonas auruginosa (ATCC-15442)* |
| *Tolypothrix tenuis* | Chloroform | 10.0±0.07 | 10.6±0.8 |
| | Methanol | 9.0±0.7 | 9.6±1.4 |
| | Water | 8.3±0.8 | 9.0±0.7 |
| | Control Gentamycin (10µg/ml) | 17.0±0.0 | 18.0±0.0 |
| *Anabaena variabilis* | Chloroform | 9.3±0.4 | 11.6±0.4 |
| | Methanol | 9.6±1.0 | 11.3±1.0 |
| | Water | 8.0±0.3 | 9.3±1.4 |
| | Control Gentamycin (10µg/ml) | 18.0±0.0 | 19.0±0.0 |
| *Cylindrospermum* sp. | Chloroform | 8.6±1.0 | 10.3±1.0 |
| | Methanol | 7.6±1.0 | 11.7±0.7 |
| | Water | 7.3±0.4 | 8.6±0.4 |
| | Control Gentamycin (10µg/ml) | 16.0±0.0 | 18.0±0.0 |

**Values are mean of diameter ± standard error (SEM) of triplicate determinations**
**Values including diameter of the paper disc (6.0 mm)**

## Discussion

Cyanobacterial extracts of *Anabaena variabilis* and *Synechococcus elongates* have shown significant antibacterial proportion towards *E.coli, Enterococcus* and *Klebsiella,* Archana *et al*., 2013). The antimicrobial activities against different bacterial strains were studied by using the strains of *Oscillatoria* sp. (Issa, A.A, 1999) and *Phormidium* sp. (Fish, S.A., 1994). Invitro antibacterial activity against acetone extract of *Spirulina subsalsa* and ethanol extract of *Oscillatoria pseudogeminata* show a high inhibitory activity (Reehana *et al*., 2012). Extracts of *Spirulina platensis* obtained by different solvents exhibited different degrees of antimicrobial activity on both Gram-positive and Gram-negative organisms have been studied (Rania *et al*., 2008). Patra *et al*., (2008) concluded that methanol extract of Cyanobacteria were active against gram negative and gram positive only. Prasantkumar *et al*., (2006) studied antimicrobial activity in organic extracts of six species of marine algae against different bacterial strains. Few studies have been made to screen Cyanobacteria from coastal region of oceanic water for the production of

antibacterial substances against bacteria by using solvent extracts of Diethyl Ether, Ethyl Acetate and Ethanol (Muftah M Zarmouh, 2010).

**Conclusion**

It is quite evident from the present investigation that the preliminary investigations of antibacterial studies of *Tolypothrix tenuis, Anabaena variabilis, and Cylindrospermum* sp. have shown antimicrobial activity in different solvent extracts like chloroform, methanol and water. In view of the present investigation it is concluded that the antimicrobial activity of Cyanobacterial strains depends on the individual solvent used for making the extracts from the different Cyanobacterial strains. Therefore, it is suggested that further detailed studies are required to confirm the impact of antimicrobial activity of crude extracts prepared from the different solvents. At the same time the isolation and characterization of the active compounds responsible for the antibacterial activities need to be evaluated further.

**Acknowledgements**

Authors are thankful to the Head, Department of Botany, Kakatiya University for providing research facilities to carry out the present investigation.

**References**

**Anand N**. 1989. Hand book of blue-green algae of rice fields of South India. Bishan Singh Mahendra Pal Singh, Dehradun.

**Archana Tiwari** and **Deepika Sharma,** 2013. Antibacterial activity of bloom farming Cyanobacteria against clinically isolated human pathogenic microbes. *J Algal Biomass Utln* .**4(1):** 83-89.

**Desikachary T.V.** 1959. *Cyanophyta.* Indian Council of Agricultural Research New Delhi, New Delhi.

**Falch B.S., Konig.G.M, Wright A.D., Sticher O., Angerhofer C.K., Pezzuto J.M.,** and **Bachmann H.** 1995. Biological activities of Cyanobacteria: evaluation of extracts and pure compounds. *Planta Med.*, **61**: 321-328.

**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.

**Frankmolle W.P., Larsen L.K., Caplan F.R., Patterson G.M.L**. and **Knubel G.** 1992. Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena laxa.* 1. Isolation and biological properties. *J. Antibiot.*, **45**: 1451-1457.

**Inderjit** and **Dakshini,K.M.M.**1994. Algal allelopathy. *The Botanical review*.**60**:182-196.

**Issa, A.A.** 1999. Antibiotic production by the Cyanobacteria *Oscillatoria angustissima* and *Calothrix parietina*. *Environ Toxicol Pharm.*, **70**: 61-63.

**Meeting B** and **Pyne JW.**1986. Biologically active compounds from microalgae, *Enzyme Microb Technol*.**8:** 388-394.

**Moore R E.** 1996. *Cyclic peptides and depsipeptides from Cyanobacteria: a review*, *J Ind Microbial*, **16**(2): 134-43.

**Muftah M Zarmouh,** 2010.Antibacterial activity of *Rivularia* species and *Oscillatoria salina* collected from coastal region of misurata, Libya. *JASMR*, **5(2):** 159-163.

**Namikoshi M** and **Rinehart K L** 1996. Bioactive compounds produced by Cyanobacteria. *J. Ind. Microbial*, **17**:373-384.

**N.Reehana,A. Parveez Ahamed** and **N.Thajuddin.**2012. Invitro studies on bactericidal effect of selected Cyanobacteria against bacterial pathogens. *Int J Med Res.*, **1(7):**345-347.

**Pankaratova,E.M**.1987. Participation of Cyanobacteria in the soil nitrogen cycle and formation of soil fertility. *In Advances in microbiology*, Nauka, Moscow, **21:**212-242.

**Patra JK,Rath SK, Jena K** and **Rathod VK** and **Thatio HN**.2008. Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) extract: A study on inhibition of Glutathione-S-transferase activity. *Turk.J.Biol*. **32:**119-125.

**Prashantkumar, P, Angadi S** and **Vidyasagar G.** 2006. Antimicrobial activity of blue green algae. *Int.J.Pharm.Sci.* **68(5):**647-648.

**Rania, M.A., Abedin Hala Taha, M.** 2008. Antibacterial and antifungal activity of Cyanobacteria and green Microalgae, evaluation of medium components by placket-Burman Design for antimicrobial activity of *Spirulina platensis*. *Global J.Biotech.Biochem*., **3(1):**22-31.

**Santra, S.C.**1993: Biology *of Rice fields blue-green algae*, Dayapublishing house Delhi.110035.