

## Antibacterial Activity of Cyanobacteria and its Extract Against Intestinal Pro-Biotics System



### Biotechnology

**KEYWORDS :** Antibacterial activity, Cyanobacteria, contaminated drinking water, pro-biotics.

**Ganesh Ramesh Pawar**

Aakriti Biotechnology, An Educational branch of AASS NGO Jharkhand

**Deepak Kumar**

98 A, Shobha Niketan, Upper Verdhaman compound, Lalpur, Ranchi-834001.

### ABSTRACT

*Cyanobacteria, the blue green algae are widely distributed throughout the world. It can be harmful if consumed through drinking water. During this study, the antibacterial activity of cyanobacteria against the pro-biotic system (useful bio-flora of animal gut) is tested and observed. The pro-bacteria are reported to cure many diseases originating due to stomach infection. The digestion of food is the source for the energy; if pro-biotic bacterial system is destroyed the effect would lead to the generation of many digestion related problems. The current work focuses on the antibacterial activity of cyanobacteria against probiotics, if used in the wild form as well as the solubility based extracted product. The destruction of gut bio-flora due to the intake of drinking water is the area of concern.*

### Introduction

Cyanobacteria are a very old group of organisms and represent relics of the oldest photoautotrophic vegetation in the world that occur in freshwater, marine and terrestrial habitats (Ghasemi N *et al* 2003). Cyanobacteria are an assemblage of gram-negative eubacteria. They are structurally diverse and widely distributed throughout the world and are later known as blue green algae (Vijaya S *et al* 2013). Cyanobacteria are characterized by their capacity to perform biological nitrogen fixation and oxygenic photosynthesis. Cyanobacteria are considered being one of the potential organisms useful to mankind in various ways. A number of important advances have occurred in cyanobacterial biotechnology in the recent years. Worldwide attention is drawn towards cyanobacteria for their possible use in mariculture, food, fuel, colorant, production of various secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals, pharmacological probes and pollution abatement. Secondary metabolites from cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects (Ritika C *et al* 2011). 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. Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetic importance. Recently, there has been an increasing interest in cyanobacteria as a potential source for new drugs. The main objectives of this study were to isolate the marine cyanobacteria and test them for their antibacterial effect.

On the flipside, cyanobacteria can prove to be harmful as it may lead to gastrointestinal symptoms such as stomach pain, nausea, vomiting, and diarrhea. The commercial application of microalgae derived compounds has received very little attention in the area of pharmaceuticals, antibiotics and other biologically active compounds. Hence, screening of Cyanobacteria for antibiotics and other pharmacologically active compounds has received interest as a potential source for new drugs (Shweta Y *et al* 2012). Various strains of Cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial and antiviral activity. The commonly applied methods of antimicrobial activity are based on the agar diffusion and results were shown as visible zones of growth inhibition.

Pro-biotics are microorganisms that offer some form of health benefit to the host - they can be found in various different foods. Probiotics are believed to play very important roles in regulating proper intestinal function and digestion - by balancing intestinal microflora. The World Health Organization's 2001 definition of probiotics is "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host." A reduction of beneficial bacteria can lead to digestive problems, such

as diarrhea, yeast infections and urinary tract infections etc.

Cyanobacteria are the major harmful algal group in freshwater environments and are recognized as a rapidly expanding global problem that threatens human and ecosystem health. The presence of high levels of cyano-toxins in drinking water can cause gastrointestinal complications, liver damage, neurological symptoms, and potentially even death. The public health impacts of chronic, low-level exposures are unknown. In the current study we checked the cyanobacteria in the wild form as well as its extract against the helpful pro-biotic bacterium system. It is globally known that the destruction of pro-biotic flora in gut leads to health hazards. Our study indicates that the antibacterial activity of cyanobacteria can affect the human health if contaminated water is continuously consumed.

### Materials and Methodology

#### Collection of samples

Freshwater samples of Cyanobacteria were collected from 6 different locations (Table 1).

#### Isolation and culturing of Cyanobacteria

The Cyanobacteria were cultured in BG 11 media (Cetinkaya G *et al* 1999), (Archana T *et al* 2013) which were further screened for the single isolate by serial dilution with continuous streaking. The isolated colonies from the plates were inoculated in the BG11 liquid media to obtain a pure culture of Cyanobacteria.

#### Extraction Procedure

The Cyanobacteria samples were centrifuged at 5000 rpm for 15 minutes. The aqueous solution was discarded. After centrifuging again, the Cyanobacterial pellets were homogenized (Ghasemi N *et al* 2003) and extracted using solvents like Acetone, Ethanol, Methanol, Xylem, Hexane and Diethyl ether for each Cyanobacterial culture (Reehana N *et al* 2012), (Vijayakumar M *et al* 2011). The extract were quantified by dry weight and re-used for further processes.

#### Pro-biotic culturing

A Pre activated pro-biotic bacteria was inoculated in nutrient broth for culturing at 37°C. Further separation of colonies performed by streaking and isolated colonies was cultured into the nutrient broth.

#### Antibacterial activity of cyanobacteria extract on agar plates

Probiotic culture of <1 Optical density 100 µl was spread on nutrient agar plates. The sterile filter disc was dipped in the extract, isolated from the cyanobacteria. The similar experiment was attempted with agar diffusion technique where bored well used to load cyanobacteria extracts.

### Antibacterial activity of direct cyanobacteria sample on agar plates

Probiotic culture of <1 Optical density 100  $\mu$ l was spread on nutrient agar plates. The sterile filterdisc was dipped in the cyanobacteria sample. The similar experiment was attempted with agar diffusion technique where bored well used to load cyanobacteria sample.

### Results

#### Isolation and culturing of Cyanobacteria

Cyanobacterial single isolated colonies were obtained by continuous streaking and inoculated in BG11 broth to obtain a pure culture (Fig1- A&B).

#### Extraction Procedure

Cyanobacterial pellet were extracted using Acetone, Ethanol, Methanol, Xylene, Diethyl ether and separation of compounds done by TLC (Thin layer chromatography).

#### Pro-biotic culturing

Pro-biotic bacterial identification done by Gram staining (Fig1- C).

### Antibacterial activity of cyanobacteria extract on agar plates

Clear zone was obtained by filter paper disc dipped in extract (Fig1-D, E), and also a clear zone was obtained by agar diffusion technique where bored well used to load cyanobacteria extracts (Fig1-F, G, H).

### Antibacterial activity of direct cyanobacteria sample on agar plates

clear zone was obtained by agar diffusion technique where bored well used to load pure cyanobacteria sample (Fig1- I).

### Discussion

Cyanobacteria is known to produce toxic compounds and globally reported for their damage to cattle and other animals. During this study, we were trying to identify the anti-bacterial activity of cyanobacteria against pro-biotic bacteria. The digestive system is home to more than 500 different types of bacteria. They help keep the intestine healthy and assist in digesting food. They are also believed to help the immune system. Some digestive disorders happen when the balance of friendly bacteria in the intestine becomes disturbed. These friendly bacteria also help to fight bacteria that cause diarrhea. More than 100 diseases are reported to be cured with treatment of pro-biotic system. Due to the contamination of cyanobacteria, most of the freshwater resources have become unsafe for drinking. The adverse effects of cyanobacteria cannot be seen to affect the early stage of human, the impact can be seen if the contaminated water will be consumed by the human population in the longer run.

As per our observation, the current work results indicate that cyanobacteria obtained from freshwater resources can damage pro-biotic system present in human/animal gut. This leads to the health problems associated with pro-biotic bacteria.

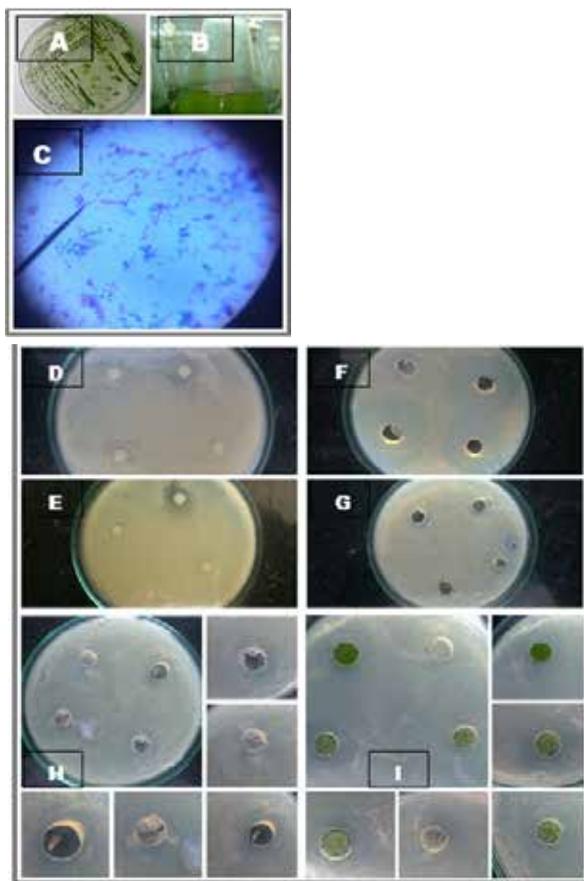
### Conclusion

It was concluded that cyanobacteria shows antibacterial activity against pro-biotic system.

**Table 1: Cyanobacteria samples collection from different geo-location**

Sr. No.	Name	Location
1.	Dr.Salim Ali lake	19°53'57.26"N 75°20'32.23"E
2.	Lonar lake	19°58'36"N 76°30'30"E
3.	Harsool lake	19°55'45"N 75°20'10"E

4.	Nehru udyan lake	19° 53' 47"N 75° 23' 54"E
5.	Sawangi lake	19°56'18"N 75°21'30"E
6.	Khultabad lake	20.009524°N 75.188799°E



**Figure: Antibacterial activity of cyanobacteria extract on agar plates**

Clear zone was obtained by filter paper disc dipped in extract (D, E), and also a clear zone was obtained by agar diffusion technique where bored well used to load cyanobacteria extracts (F, G, H). Cyanobacteria culture and strain isolation by streaking (A, Band C).

### References

1. Archana T and Deepika S (2013), Antibacterial Activity of Bloom forming Cyanobacteria against Clinically Isolated Human Pathogenic Microbes. *Journal of Algal Biomass Utilization (JABU)* 4 (1): 83-89.
2. Carmichael W.W. (1992), A review: cyanobacteria secondary metabolites the cyanotoxins. *Journal of applied bacteriology*. 72: 445-459.
3. Cetinkaya G, Ayse E and Olcay O (1999), Isolation and Abundance of Unicellular Cyanobacteria from Mosquito Development Sites. *Turkish Journal of Biology* 23: 451-456.
4. Codd GA, Lindsay J, Young FM and Metcalf JS (2005), Cyanobacterial toxins: risk management for health protection. *Journal Toxicology and Applied Pharmacology* 203: 264-272.
5. Ghasemi Y, Tabatabaei Y, Shokravi S, Soltani N, and Zarrini G (2003), Antifungal and antibacterial activity of paddy-fields cyanobacteria from the north of iran. *Journal of Sciences* 14(3): 203-209.
6. Hunter PR (1991), Human illness associated with freshwater cyanobacteria (blue-green algae). *Journal of Medical Microbiology* 36: 301-302.
7. Madhumathi V, Deepa P, Jeyachandran S, Manoharan C and Vijayakumar S (2011), Antimicrobial Activity of Cyanobacteria Isolated from Freshwater Lake. *International Journal of Microbiological Research* 2 (3): 213-216.
8. Reehana N, Parveen A and Thajuddin N (2012), In vitro Studies on Bactericidal

- Effect of Selected Cyanobacteria against Bacterial Pathogens. *International Journal of Microbiological Research* 1(7): 345-347.
9. Ritika C, Silambarasan S and Abraham J (2011), Biodiversity of Marine Cyanobacteria and its Antibacterial Activity. *Journal of Biotechnology and Biotherapeutics* 1: 4.
  10. Senthil K, Sivasubramanian V and Mukund S (2013), Antimicrobial and Antifungal activity of extracts of *Phormidium fragile* Gomont. *Journal of Algal Biomass Utilization* 4 (3): 66-71.
  11. Shweta Y, Rajeshwar S and Madhu T (2012), Antimicrobial Activity of Some Cyanobacteria. *International Journal of Pharmacy and Pharmaceutical Sciences* 4: 3.
  12. Stewart I, Seawright A, Shaw R (2008), Cyanobacterial poisoning in livestock, wild mammals and birds. *Advances in Experimental Medicine and Biology* 619: 613-637.
  13. Vijaya S and Ashok V (2010), Antibacterial Activity of Cyanobacterial Species from Adirampattinam Coast, Southeast Coast of Palk Bay. *Current Research Journal of Biological Sciences* 2(1): 24-26.