Assessment of antimicrobial properties of Cyanobacteria collected from various places of Bilaspur town,Chhattisgarh.

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ABSTRACT

The objective of the study was to test the antimicrobial property of cyanobacteria with various solvent and crude extracts such as (methanol, chloroform, aqueous, ethyl acetate)two pathogenic bacteria, *Pseudomonas Aeruginosa*-(ATCC 27853) and *Staphylococcus aureus*-(ATCC 25923) are used for antimicrobial activity. Cyanobacterium strains *Anabaena iyengrii*, *Microcystis aeruginosa*, *Oscillatoria princeps* were collected from the Bilaspur town of Chhattisgarh during rainy season and maintained in (BG 11 medium).the antimicrobial activity was determind by agar disk diffusion method. In which the culture extract of *Anabaena iyengrii* was shown with significant antibacterial activity (5.2 ± 0.62mm) in the solvent of crude extract against , *Pseudomonas aeruginosa*-(ATCC 27853) under observation . thus the genus *A. iyengrii* proved to be more potential in bioassay studied against selected bacterial starin.

INTRODUCTION

Cyanobacteria (Gr.Kyanos, blue + Gr. Chloros, green+Gr. Bacteria,being) have been included in blue green algae of class –Myxophyceae by majority of Phycologists . Cyanobacteria are common inhabitants of water logged area as in fresh water, sea water, salt water throughout the world . Certain non heterocystous form of cyanobacteria can dominate phytoplankton within water reservoirs and also produce potent toxins .Cyanobecteria are gram positive photosynthetic bacteria which perform oxygenic photosynthesis.

Cyanobacteria play spectrum of remarkable roles in the field of energy production, biofertilizer, human food, animal feed, polysaccharides, biochemical and pharmaceuticals such as antibiotics (Richmond, 1990; Skulberg, 1994; Flash *et al.* 1995) Cyanobateria have been identified as an important agent for the control of various pathogens (Hewedy *et al.*, 2000). Cyanobacteria have many advantages compared to antifungal drugs used against plant pathogens, they are easy to cultivate (Allen, 1968) requiring simple media and light, produce antimicrobial metabolites and safe for environment compared to synthetic fungicides. Inconsistencies between the approaches are evident, although taxonomy of filamentous cyanobacteria with heterocysts as represented by *Anabaena cylindrical*, still hold true. There are an estimated 150 genera of cyanobacteria containing approximately 2000 species, of which around 46 have been reported as being toxicogenic (Hitzfeld *et al.*, 2000; Ernst *et al.*, 2006). Importantly, the genera and species which comprise problematic cyanobacteria are generally well recognized.

Screening of cyanobacteria for antibiotics and other pharmacologically active compounds has received considerable attention during the past few decades. Extensive screening programs from cyanobacterial biomass or from the laboratory cultures have led the discovery of novel compounds with antimicrobial, antineoplastic and cytotoxic activities. [Kretlow et al] investigated the hydrophilic and lipophilic extracts of cyanobaterial strains from fresh and brackish water, and two water blooms, from the Baltic Sea for their antibiotic activities.

Many Cyanobacteria produce compounds are generally considered to be secondary metabolites that are not essential for general metabolites or growth of the organism and are present in restricted taxonomic groups. Microcystis, Nostoc, Anabaena and Oscillatoria turn out an excellent kind of secondary metabolites.

The treatment of diseases caused by bacteria and fungi is becoming an issue of concern, due to growing emergence of microorganism strains resistant to drugs and opportunistic fungi that cause serious infection in humans. Microbial resistance is a genetic phenomenon, in which the microorganisms have genes that encode biochemical mechanisms which prevent drug actions. It may be caused by the mutations in the reproductive process of the microorganisms or by

Cyanobateria are a large group of oxygenic photoautotrophic bacteria and, like plants and algae, can capture CO_2 via the Calvin-Benson cycle and convert it to a suite of organic compounds. They are important primary producers of organic materials and play significant roles biochemical cycles of carbon, nitrogen, and oxygen. Cyanobacteria are well suited for synthetic biology and metabolic engineering approaches for the phototrophic production of various desirable bimolecular, including substances with cytotoxic, antifungal, antibacterial and antiviral activities. Lipids, carotenoids, pigments, vitamins, and aromatic compounds are also found in cyanobacteria. Lipids (accumulated in the thylakoid membranes) are associated with high levels of photosynthesis and rapid growth rate and are of particular interest, since they can be used as lipid feedstock for biodiesel production. Microalgae accumulate large amounts of lipids as reserve material, but only in conditions of stress and slow growth. Thus, Cyanobacteria have a natural advantage to produce lipids in high speed growth.

Recently, biodiesel derived from microbial biomass is attracting much attention and investment and can meet the demand necessary for biodiesel supply without commitment to the food chain. The presence of double bonds in the FAs from cyanobacterial lipids is related to their morphological complexity. It has been reported that unicellular forms are characterized by the presence of mono and polyenoic acids.

Although, Cyanobacteria have several advantages for biofuel production, including easy genetic manipulation to increase photosynthetic efficiency, fast growth and high lipid content in the biomass, there are few specific studies on their use as a source of lipids. In this context this study aims to evaluate the potential of five Cyanobaterial strains as producers of lipid feedstock for the synthesis of biodiesel. Biomass and lipids productivities and FAs profiles were evaluated.

Keeping in view the fact is mentioned above, the present course of work has been planned to access the antimicrobial properties of Cyanobacteria different water logged areas of Bilaspur town of Chhattisgarh and investigation have been done for the same to achieve the following objective.

1. Assessment of exo-toxic properties and antimicrobial behavior through standard methods.

Materials and methods:-

Source and sites of Cyanobacteria were selected in proposed area for present investigation. Survey of spots and sampling of Cyanobacteria was performed by graphical division whole area into several zones. The samples were collected during consecutive day of the month from all sources/spots.

Survey of the Cyanobacterial flora:-

A survey of water-logged spots have been made within Bilaspur town with surroundings and the study sites have been marked such as ponds/tanks, ditches/pasture lands and paddy fields. Survey on Chhattisgarh state has been done recently by Shrivastava, 2000; Tiwari *et al*.,2004; Shrivastava *et al., 2005, 2007*. Bilaspur district of Chhattisgarh state, as well as the adjoining division of Kabirdham, Durg, Raipur, Balodabazar, Janjgir, Korba, Korea and state of Madhya Pradesh, is famous for their well distributed paddy-fields, and the cyanotoxin producing Cyanobacterial flora.

Collection and Identification of Cyanobacteria:-

Samples were collected from different sampling sites, i.e. paddy fields, ponds and tanks, pasture lands and ditches, following the sampling method. Organisms were collected in different polythene bags and in autoclaved bottles treated with 10% HCl along with spot water. The samples were used for identification and enumeration.

Identification:-

After the survey of the study sites, in view of Cyanobacterial flora, a number of heterocystous and non-heterocystous Cyanobacterial forms were identified and isolated in the form of pure culture. For the selection of the experimental material all Cyanobacterial species were critically examined for their relative efficiency to forms discrete and conspicuous colonies on agar medium for facilitating to produce colonel population. Morphological observation of Cyanobacterial forms was made through compound light microscope and Micro Imaging Photography system (MIPS), Model no. ML-TR (Olympus). Based on characterization, systematic identification of Cyanobacterial species were performed using morphological variation and taxonomical approaches according to (Desikachary, 1959) and (Anand, 1989).

Isolation of cyanobacterial strains:-

For the culture of cyanobacteria, collected from the fields, synthetic nutrient media, i.e. Allen and Arnon, BG-11 and Chu-10 and (vi) MN, formulated by Allen and Arnon (1955), Rippka *et al*., (1979), and Chu, (1942), modified by Gerloff *et al.,* 1950 and VI) Rippka *et al.,* (1979), were used. autoclave. The pH of the

medium was adjusted at the post autoclaved stage by the addition of dilute N/10 HCl (or NaOH) as required for the media.

Screening of Exo-Toxin Releasing Cyanobacteria:-

During the survey and collection of entire zones 22 Cyanobacyerial strains were collected and identified, out of which common, frequent and most prevalent strains were selected for analysis of toxic nature and have been observed during the present course of investigation in paddy fields, ponds and other study sites of Bilaspur division of Chhattisgarh. Different toxins producing species which were found like as, *Aphanocapsa* species, *Aphanothece* species, *Anabaena* species, *Microcystis* species, *Nostoc* species, *Oscillatoria* species, etc.

Preparation of Crude Extract:-

- Samples were kept in 4^{o}C during storage and transit. It is then cloned for laboratory culture intensively under sterile condition using Chu-10 (26^{0}C, 0.003% CO2, 24 hr light) medium by standard procedure adopted after Vishampayan *et al., (1984), (1992).* Samples were concentrated with the help of centrifugation at 10,000 rpm for 15 min. and were filtered through a 0.45 **um glass** fiber filter, dried in an oven at 55^{0}C.
- Dried cell mass – 100mg/100ml (w/v) of sample were extracted with 75% methanol, for 4-5 hrs. then centrifuged at 5000 rpm for 7 min. the supernatant was separated in fresh glass vials and filtered with 0.45 um pore size and methanol was evaporated different dilutions were prepared for cyan-toxicity test in- vitro.

Assessment of Toxic Nature of Cyanobacteria:-

Antibacterial bioassay:-

Overnight cultures (at 37^{0}C for 24hr) of each bacterial strain (*Pseudomonas aeruginosa and Staphylococcus aureus)* were swabbed with sterile cotton on the surface of Nutrient Agar plates. The antibacterial successibility was screened using the filter paper disc (5mm in diameter) diffusion method. Disc was saturated with 50ul (100mg/100ml) of the crude extract of *Anabaena iyengrii*, *Microcystis aeruginosa and Oscillatoria princeps and* dried under laminar air flow. Then the dried discs were placed on the surface of the swabbed plates. The plates were incubated for 24 hr at 37^{0}C. Solvent control was performed in each case. After 24 hr, areas of inhibition of bacterial growth were observed as clear zone around the well and diameter of inhibition was measured in mm. Different Concentrations (25%, 50%, 75% and 100%) of extracts were used and results were observed. The minimum inhibitory concentration against the aforementioned strains was compared to standard and commercially available antibiotic disc – Kanamycine – 30 mcg and Amoxycillin 30 mcg.

growth pattern was observed. Redial diameter was recorded twice perpendicularly after 96 hrs incubation using a transparent mm ruler. Percentage inhibition of Mycellial growth was calculated.

% Mycellial inhibition =100X Mycellial growth (control) – Mucellial growth (treatment)

Mycelial growth(control)

RESULT:

Microcystis aeruginosa

Colonies are young round or slightly longer than broad solid. When ol becomes clathrate with district hyaline colonial mucilage, cells occur 3-7 µ in diameter spherical with gas vacuole.

Anabaena iyengerii

It was reported from paddy fields of Bilaspur district. Trichome is single and irregularly curved 5.2 µ broad and cell with conical and rounded apex. Each cell is shaped as long as brood barrel shaped heterocyst, spores ellipsoidal in short chain.

TABLE:- Toxic behavior of crude extracts of *Microcystis aeruginosa and Oscillatoria boryana and Staphylococcus aureus and its comparison* with standard antibiotics (Mean± SD).

Cyanobacterial Crude extracts and Standard Antibiotics		Concentrations	Zone of inhibition (mm)	
			Pseudomonas aeruginosa-ATCC-27853	Staphyloccus aureus-ATCC 25923
		0 %	00	00
Crude Extracts	*Anabeana iyengrii*	25 %	00	00
		50 %	2.8±0.21	1.9±0.62
		75 %	3.9±0.33	3.4±0.38
		100 %	5.2±0.62	4.9±0.42
	Microcystis aeruginosa	25 %	8.20±0.47	7.70±0.62
		50 %	12.5±0.61	10.3±0.40
		75 %	18.6±0.52	16.2±0.65
		100 %	19.5±0.73	20.0±0.13
	Oscillatoria princeps	25 %	7.80±0.43	8.00±0.55
		50 %	12.8±0.29	10.3±0.46
		75 %	16.6±0.67	15.2±0.28
		100 %	18.5±0.83	20.0±0.47
Antibiotics	Kanamycin	30mcg./ disc	--	13.5±0.30
	Amoxicillin	30mcg./ disc	7.00±0.73	26.0±0.37

Conclusion:-

Through the present course of investigation, it became clear that area of Bilaspur (Chhattisgarh) has proper habitat of Cyanobacterial flora as a rich source of toxin producers.. Thus Cyanobacterial extracts can be used for the treatment of infectious diseases caused by resistant

bacteria. It may represent the new source of anti- microbial property with stable, biologically active compounds that can establish a scientific base for the use in modern medicine. It can be extended for future investigation in the field of pharmacology, phytochemistry and other biological actions for drug discovery. The co-occurrence of toxic compound in one single organism is a source of numerous future studies on bioactivity of metabolites from cyanobacteria. This approach is necessary for a better understanding of the ecological role of cyanobacterial metabolites. This concluded that further isolation and characterization of cyanobacterial metabolites are responsible for anti- microbial properties which may lead to the formulation significant pesticides of biological origin.

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