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Isolation and Characterization of Cyanobacteria from Ponds of Muzaffarpur District Bihar India

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Abstract
In this investigation Cyanobacterial members were found in all selected locations of Muzaffarpur district, Bihar state. An attempt has been made to isolate Cyanobacteria from ponds of Muzaffarpur district Bihar State. These are characterized based on their morphological character by using standard literature and key points. Cyanobacteria are the most important group of nitrogen fixing organism. Among algae, the cyanobacteria are considered to be very valuable in agriculture and industries. To initiate the study water sample were collected in Erlenmeyer flasks and brought to the laboratory. Cultures were maintained and periodical observations were made for isolation and identification of different species of Cyanobacteria that is useful for their identification by Morphological and Biochemical studies of some Cyanobacterial strains, of Ocillatoria princeps , Microcystis aeruginosa, and Riluveria species, Gloeocapsa magma.

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INTRODUCTION

The city of Muzaffarpur lies on the southern banks of the Burhi Gandak River and covers a total area of 26.68 km. The city is presently divided into 40 wards. Its geographical coordinates are 26^{o}07 Latitude and 85^{o}27 Longitude and its altitude from the mean sea level is 187 ft. Experimental Ponds field areas of the Muzaffarpur Municipal Corporation, B.R.A.Bihar University campus and Tirhut as well as the block of Kanti, or the Motipur Nagar prishad has been chosen as the study area under Muzaffarpur.

Cyanobacteria is a group of cosmopolitan, gram negative, oxygenic, photosynthetic, prokaryotes which grow and multiply at the sample expense of water, light and air (Fay, 1983) and perform two biologically important functions such as carbon and nitrogen fixation and thus enriching the soil fertility with humus and nitrogen content. (Singh et al., 2014). They are ubiquitous in nature and sometimes found in extreme habitats too (Hoffman, 1989; Whitton and Potts, 2000). During their long and slow evolution, they have achieved huge diversity both in morphology and genetics, ranging from simple unicellular organisms to complex filamentous organisms (Whitton, 1992). A few can fix atmospheric nitrogen in its usable form and

have been shown to be agriculturally important as bio fertilizer, particularly in tropical ponds field (De, 1939; Stewart et al., 1987; Singh et al., 2014). Ponds field provide a very congenial condition for abundant growth of N_2-fixing Cyanobacteria (Nayak et al., 2001; Whtitton., 2000, Srinivas et al.,2016). They are known to maintain the homeostasis of ponds field as a sustainable system (Bhattacharya, 2013).

MATERIAL AND METHODS

Study Area:
Experimental Ponds field areas of the Muzaffarpur Municipal Corporation, B.R.A.Bihar University campus and Tirhut as well as the block of Kanti, or the Motipur Nagar prishad has been chosen as the study area under Muzaffarpur. The photographs of different pond areas are given below in following plates-I and II.

Plate-I-Place of sample Collection- B.R.A.U. Bihar University Campus

Plate-II-Place of sample Collection: Ratanpura, Motipur

Laboratory Study:
From these ponds we have planned to collect the Cyanobacterial sample specimen. Thereafter, collected cyanobacterial samples were planned to isolate in the laboratory condition. After isolation of pure culture, cultures were subject to identification and growth characterization as per the protocols similar to the design of experiment of objective.

Culture Media Preparation:
For the Isolation, growth, culture characteristics of different species of cyanobacteria, in this research article we have taken BG 11 Media. Photographs of BG 11 media are given below by following plates III, IV, V & VI.

Isolation and Screening of Cyanobacteria
The water samples were inoculated in Erlenmeyer flask having cyanobacterial culture medium (composition in gm/lit: sodium nitrate 0.1gm, dipotassium phosphate 0.25 gm, magnesium sulphate 0.051 gm, ammonium chloride 0.0051gm, calcium chloride 0.005gm and ferric chloride 0.0005 gm) and incubated at room temperature under continuous dark and sunlight period for 15-20 days, growth from the incubated flask were spread on the Cyanobacterial culture plate and incubated at room temperature under continuous dark and sunlight period for 15-20 days as described by scientist (Mayur Gahlout et.al 2017). Isolated colonies were observed under the compound microscope for morphological characterization. Characterization of BGA was carried out based on some morphological features such as thallus color, thallus morphology and dimension, size of heterocyst, vegetative and reproductive cells. As the most of Cyanobacteria were associated with other microorganisms, these must be purified from any contaminants, hence exposed to different trials for purification. However, washing, ultra violet irradiation and mercuric chloride treatments were the most effective method for obtaining cyanobacteria cultures free from bacteria, while the other methods gave some success for killing bacteria on one side and some failure of the other side, which could be

lethal for cyanobacteria themselves These isolates were examined for their morphological and cultural characteristics on BG-11 media. Photographs of BG 11 media, pure culture on media & three days of isolation of pure culture are given below by following plates III, IV, V & VI.

Plate-III-Culture in Room Temperature

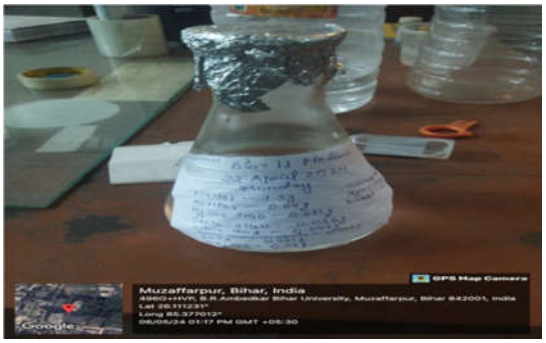

Plate-IV-Sample of BG-11 Media

Plate-V-Sample of Pure Cyanobacterial Culture

Plate-VI-Enrichment of Cyanobacterial Culture (3rd day)

Culture condition of *Oscillatoria, Rivularia, Microcystis, Gloeocapsa* species in Laboratory

RESULT

In this research work we have identified Morphological structure with its features of four genera of Cyanobacteria on pure culture of BG11 media. The specimen samples were taken from different ponds of Ratanpura, Motipur and few places under the campus of B.R.A. Bihar University of Muzaffarpur Bihar shown in **Table-1.** After the observation of so many growth media of pure culture and culture characteristics and Morphological features, there are four species of Cyanobacteria have been identified. Name of these isolated & identified species are 1. *Oscillatoria princeps* 2. *Gloeocapsa magma* 3. *Microcystis aeruginosa* 4. *Rivularia species.* The Morphological structures of these species of Cyanobacteria are shown below by following plates: VII, VIII, XI & X.

Table 1:

Name of the Genera	Family	Order	Class	Place of Collection
Oscillatoria	Oscillaoriaceae	Oscillatoriales	Cyanophyceae	Motipur Pond, Muzaffarpur
Gloeocapsa	Chroococcaceae	Chroococcales	Cyanophyceae	B.R.A.Bihar University Campus, Muzaffarpur
Microcystis	Microcystaceae	Chroococcales	Cyanophyceae	B.R.A.Bihar University Campus, Muzaffarpur
Rivularia	Rivulariaceae	Nostocales	Cyanophyceae	B.R.A.Bihar University Campus, Muzaffarpur

Plate-VII- *Oscillatoria princeps*

Plate-VIII- *Rivularia species*

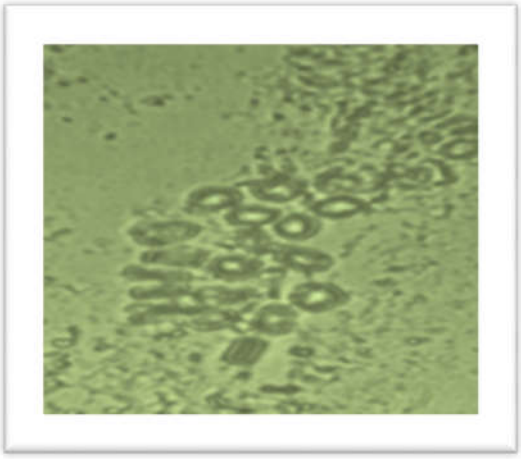

Plate-X- *Gloeocapsa magma*

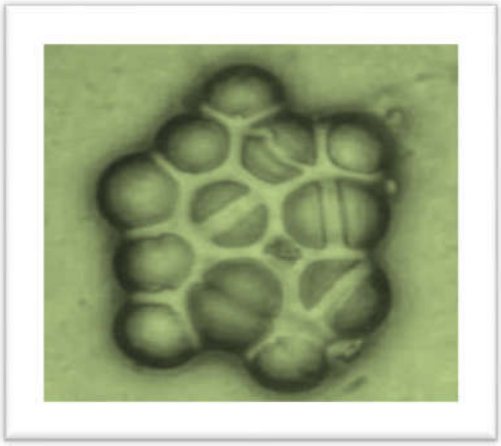

Plate-IX- *Microcystis aeruginosa*

Microscopic images of few species of Cyanobacteria isolated from the campus of Bihar University and Ratanpura, Motipur Pond of Muzaffarpur,Bihar

DISCUSSION

The Ponds field ecosystem provides an environmental favorable for the growth of Cyanobacteria with respect to them requirements for light, water, high temp and nutrient availability (Roger et al., 1993). Cyanobacteria are found all over the world in environmentally as diverse as Antarctic soil and volcanic hot spring often where no other vegetation can exist. (Knoll, 2008). Some Cyanobacterial strains i.e., *Aulosira fertilissima*, *Anabaena variabilis*, *Nostoc muscorum*, and *Tolpothrix tenuis* are being used in algal bio fertilizer technology (Kaushik, 2014). Most of the nitrogen of Cyanobacteria is released only after decomposition and autolysis (Martinenz, 1984). The majority of Cyanobacterial strains release an insignificant amount of ammonia during their growth period (Martinenz, 1984). Search for continuous ammonia secreting Cyanobacteria strains are one of the primary goals of plant biologist. The Cyanobacteria strain was isolated, characterized, and studied for ammonia secreting properties. Cyanobacteria are very resistant to extreme environmental condition and even they tolerate to high temperature up to 50^{o}C. They are increasing importance in frontier areas of biotechnology.

CONCLUSION

In this research it has been found that we have observed four species of Cyanobacteria and these are *Ocillatoria princeps, Microcystis aeruginosa, and Riluveria species, Gloeocapsa magma* . These species are known to have significant ecological and economic importance, contributing to nutrient cycling in Agricultural field of Muzaffarpur & its surrounding areas and exhibiting potential for various biotechnological applications in agricultural field and other places. This study aims to explore the diversity of Cyanobacteria in the aquaculture ponds of Motipur, assess their ecological significance, and analyze their potential utility in environmental and industrial applications.

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