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Isolation, Identification, Purification and in Vitro Axenic culturing of Cyanobacterial Strains from Godavari River, Nanded, Maharashtra

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Abstract: ***Cyanobacteria (Cyanobacteria/Cyanophytes/ Cyanoprokaryotes) are a diverse group of oxygenic photosynthetic prokaryotes. Cyanobacteria are known to produce metabolites with diverse biological activities, such as antibacterial, antifungal and antiviral, herbicides, anticancer and antiplasmodial activity. The main function of cyanobacterial microalgae is to fix the atmospheric nitrogen. In present study we have isolated and identified two cyanobacterial strains from Godavari river basin, Nanded. The culturing of axenic form of cyanobacterial strains; Microcystis sp. and Oscillatoria sp. were done by using selective medium BG-11. Antifungal agents such as Streptomycin and Flucanozole were proved effective to eradicate microbial contamination at concentration of 80µg/ml. The axenic cultures were maintained for further studies.***
Keywords: Cyanobacteria, axenic cultures, purification, BG-11.

I. INTRODUCTION

Cyanobacteria are also known as 'Blue green algae' or 'cyanoprokaryotes'. Cyanoprokaryotes are unique among microorganisms due to having chlorophyll a and their ability to made photosynthesis [1]. Due to their high capacity for morphological and physiological adaptations to different environments, both algae and cyanobacteria often act as pioneer microorganisms in terrestrial ecosystems [2], [3]. In the context of identification and classification, cyanobacteria are complex group [4], because the morphological characteristics conventionally used for their order and family level identification, as well as the type of thallus organization, trichome polarity, the presence and type of branching, the presence of sheaths, heterocysts or akinetes, cell and trichome size etc., seemed to appear and disappear several times during their long term and intricate evolutionary history [5], [6], [7]. These microalgae are the cell organisms that can easily grow in both fresh water and sea water. In addition to high levels of provitamin A, dried microalgae can provide various other nutrients including proteins minerals and antioxidants. World production of consumable algae and algae products to be used as dietary suppliments, food additives, functional foods and medicines has reached thousands of tons per year [8], [9]. Earlier several studies were carried out to isolate different endemic algal species for their important secondary metabolites [10], [11], [12]. Spencer used penicillin to purify cultures of Phaeodactylum. Since then, antibiotics have taken their place among the standard techniques for purifying algal and protozoan cultures [13]. Preparation of axenic culture of microalgae is required to obtain a viable culture of a single species, free of other species or "Contaminants" [14]. Studies on algae for over a century were on understanding their structure and reproduction and several treatise [15], [16], [17]. It has been suggested that the techniques normally used to isolate cyanobacteria may severely limit the number of cyanobacterial species which can be readily cultured [18]. Collection, sampling, and preservation techniques must be optimized to grow a high quality microalgae sample. Three important isolation techniques are commonly used for microalgae collection: 1. Streaking 2. Serial dilution 3. Single-cell isolation [19]. Thus, the aim of this study is to isolate, identify and maintain pure and axenic cultures of cyanobacteria in-vitro.

II. MATERIALS AND METHODS

A. Collection of water sample

Water samples were collected from Godavari river basin, Nanded in Maharashtra lies between in latitude 19^0 8'7.9152" and longitude$77^0$19'25.7880". The collection of water sample was done with the help of laboratory tools like foresceps, spatula, droppers and 1000ml bottles. The collection of water sample was done at morning time. For identification fresh microalgal samples were used. 4ml of samples were preserved in 4% formalin. Microphotography was done by mounting temporary slides in 10% glycerin. Microphotography was performed Digi camera with light microscope. Identification of samples was carried out with the help of standard literature [17], [20].

B. Isolation of microalgae

Samples collected from study site were enriched in BG-11 liquid medium. Then they were 10 fold serially diluted to obtain diluted mixed culture of cyanobacteria. Then 10^{-8} and 10^{-5} dilutions were selected for isolation of cyanobacteria. With the help of micropipette 100ul mixed microalgae solution from both dilutions were spread on plates of solidified BG-11 media. The BG-11 was used as isolation media. It was sterilized by autoclaving at 121°C for 20 minutes. The sterile media were cooled to room temperature and microalgae inoculation was performed aseptically in a laminar air flow hood. Replications of streaking were done until obtaining single strain of cyanobacteria. The microalgae cultures were maintained in solidified BG-11 media containing agar (Himedia Laboratories, Mumbai) at a concentration of 1.0% w/v. The liquid cultures were sub-cultured every two weeks and agar plates every four weeks once.

C. Purification of cultures

After obtaining single strain culture of cyanobacteria it is needed to make it free from other fungal and bacterial contaminants. For this purpose cyanobacterial cultures regularly observed under microscope. To overcome the contaminants antibiotic dose of 80ug/ml streptomycin and Flucanozole was added to BG11 media to get pure and axenic cultures of cyanobacteria.

D. In-vitro axenic culture

Axenic cultures of cyanobacteria obtained by dilution and plating on BG-11 media under controlled conditions of light and temperature. Addition of antibiotics was continued to the cultures to keep it in pure form. Preservation of cultures was done under freeze temperature at 4^0C.

E. Incubation conditions

The all microalgae cultures were incubated at constant temperature of 30°C ±1°C under illumination of white fluorescent lamps. All the experiments were carried out in Erlenmeyer flasks.

F. Morphological identification

Morphological identification of cyanobacteria was carried out by using light microscope and standard methods described by Prescott[17] and Desikachary [20].

III. RESULTS AND DISCUSSION

A. Isolation and purification of cyanobacterial strains

Water samples from Godavari River, Nanded were cultured on BG-11 media. Single colonies were picked up from BG-11 media, diluted and streaked on fresh agar BG-11 media plates. No fungal and bacterial observed was observed in the culture. To eradicate these contamination antiobiotics such as Streptomycin and flucanozole dose of 80ug/ml were used. As a result we obtained pure and axenic cultures of cyanobacteria strains.

B. Identification by Light microscopy

Water Samples were collected during the morning of the day in clean polythene bags and in sample bottles. These microalgal strains identified by microphotography. Microphotography was done by of Digi camera with light microscope. Identification of samples was done with the help of standard literature (Desikachary, 1959).

C. Axenic cultures of cyanobacterial strains

Axenic culturing of two cyanobacterial strains was successfully done. These two cyanobacterial cultures were Microcystis aeruginosa, Kuetz (Fig-1), and Oscillatoria curviseps, Agardh (Fig-2).

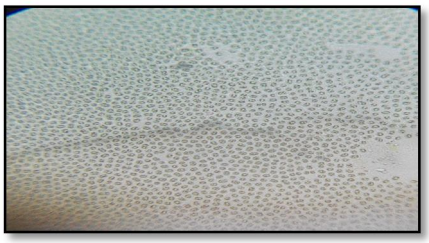

Fig-1: Microcystis aeruginosa, Kuetz

D. Microcystis Aeruginosa, Kuetz (Fig-1)

Class- Cyanophyceae; Order- Chroococcales; Family- Microcystaceae; Genus- Microcystis; species- aeruginosa. Microcystis aeruginosa is a unicellular, planktonic freshwater cyanobacterium. The existence of intracellular structures, the gas vesicles, provides cells with buoyancy. These hollow, gas-filled structures can keep Microcystis cells close to the surface of water body, where there is optimal light and oxygen for growth. Thus, when the water column is stable, the colonies can accumulate at the water surface and form surface water blooms. Cells range from 2.61 to 5.40µm in diameter, and can be either ovoid or spherical in shape.

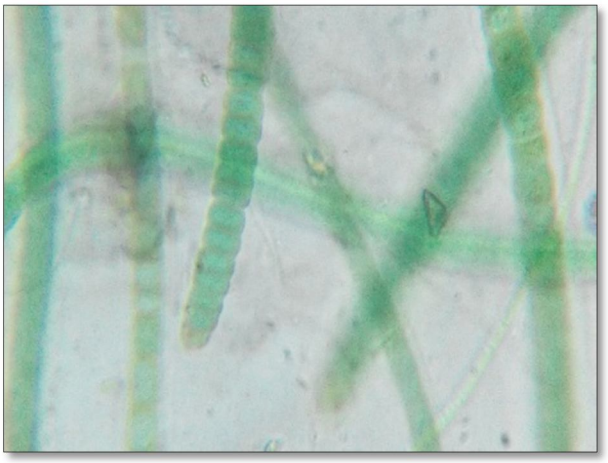

Fig-2: Oscillatoria Curviceps, Agardh

E. Oscillatoria Curviceps, Agardh (Fig-2)

Class: Cyanophyceae; Order: Oscillatoriales; Family: Oscillatoriaceae; Genus: Oscillatoria; species:*curviceps* In the form of pure culture *Oscillatoria* appears like bluish green sticky mass which settles down in the media. In this algae trichomes are blue green, more or less brownish, violet or reddish, mostly forming a thallus, mostly straight, not constricted at the cross wall, 16-60 µ broad, commonly 25-50µ, blue green to dirty green, slightly or briefly attenuated at the apices and bent; cells 1/11-1/4 as long as broad, 3.5-7 µ long; end cells flatly rounded, slightly capitates without or with slightly thickened membrane (Desikachary 1959).

IV. CONCLUSION

These findings concluded that isolation of microalgae such as cyanobacteria can be carried out using serial dilution technique which proved successful. While plating or inoculation of old cultures onto the BG-11 media was a convenient and it will give rapid way to evaluate contamination. Examination of cultured strains was best way to overcome of bacterial and fungal growth. The axenic cultures of two cyanobacterial strains of *Microcystis aeruginosa*, Kuetz (Fig-1) and *Oscillatoria Curviceps*, Agardh (Fig-2) have been maintained by using antibiotics streptomycin and flucanozole at specific concentration. So, we conclude here that these axenic cultures can be used for biotechnological applications.

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