

# Isolation and Pure Culturing of Elite Strains of Blue Green Micro Algal forms for Biofertilizer as initial Cultures from Swami Ramanand Teerth Marathwada University Nanded, Maharashtra

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**Abstract:** Algae are photosynthetic thallophytes or lower plants which grow in water or on soil saturated with water. Blue green algae are distributed worldwide to enhance the fertility of many agricultural ecosystems. Blue green algae are a group of microalgae that can fix the atmospheric nitrogen. Isolation of these cyanobacteria from natural sources in pure form is essential step for their efficient use as biofertilizer. In present investigation three species of blue green microalgal forms are isolated from natural fresh water and soil habitats of S. R. T. M. U. Nanded. The isolation of pure cultures were done by selecting a single colony from mix cultures grown on selected media like BG-11, bold basal media, ASN III media as different BGA strains can grow on different media. The same media in solid form is used for further purification and subculturing. Pure culturing was done by adding streptomycin as an antibiotic and fluconazole as an antifungal agent. The pure cultures were then transferred in solid and liquid media for further studies. Special observations were made on their growing pattern on solid as well as in liquid culture.

**Keywords:** Blue green algae, pure culture, elite strains, microalgae

## 1. Introduction

Microalgal biodiversity in of blue green algae or cyanobacteria in fresh and marine habitat has been investigated in India and abroad by many workers. The pioneer workers are Allen and Stanier (1967) who have cultured different aquatic blue green microalgae in laboratory. Abdo (2005) from Egypt and Rai (2011) from Nepal have worked on biodiversity of fresh water microalgae. In India fresh water blue green algal biodiversity has been done by Arulmurgan (2011) from Chennai, Baruah (2009) from Assam, Mahadik and Jadhav (2013) from Ujani, Dalal and Nisal (2012) from Maharashtra, Ingle (2012) from Satara, Narwade (2014), Mulani and Sonule (2015) from Marathwada.

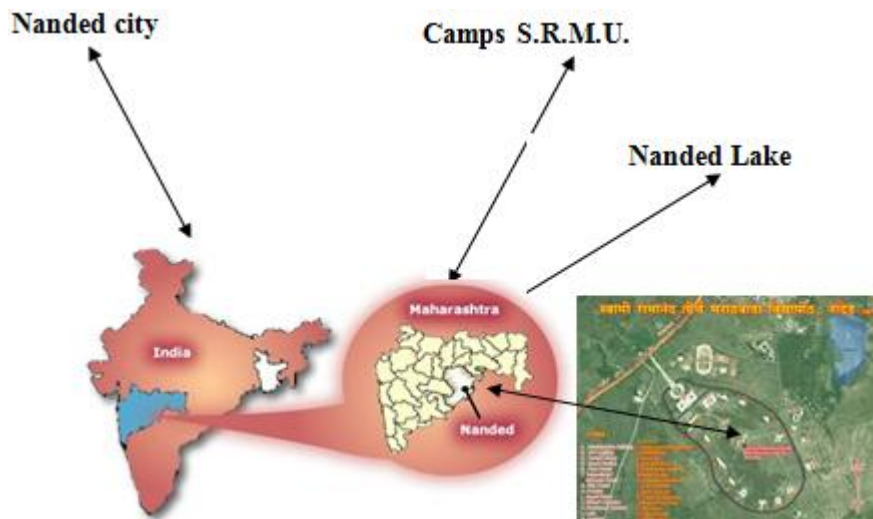
The microalgal growth in pure culture is mainly determined by biotic and abiotic factors. Abiotic factors are like light, temperature, the concentration of nutrients, oxygen, and CO<sub>2</sub>, P<sup>H</sup> and mineral composition of the medium etc. while biotic factors influences algal growth in culture media like viruses, fungi and bacteria (Mata *et.al.* 2010). On microalgal

blue green pure culture were isolated by Allen and Stanier in India, Maharashtra Mulani – UGC project at Mumbai.

It is essential to obtain microalgae in pure culture as these strains have potential for biodiesel production and as a biofertilizer enhancing the agricultural productivity. Traditional isolation techniques include the use of a micropipette for isolation under a microscope or cell dilution followed by cultivation in liquid media or agar plates (Duong *et. al.* 2012). Algae can convert solar power into chemical energy and fix atmospheric nitrogen. Blue green algae can reduce CO<sub>2</sub> to power consuming biomolecules like carbohydrates, proteins, lipids and triglycerides (Varfolomeev *et.al.* 2010).

## 2. Materials and Methods

**Study Area:** Present investigation was carried out at the campus of Swami Ramanand Teerth Marathwada University Nanded lies between 19°06'00.3"N to 77°17'15.6"E of eastern side of Nanded Latur highway covers almost 550 acres (fig 1).



### Collection of Samples

Composite soil samples were collected from different selected sites like lake area, botanical garden, vicinity of school of life sciences. The soil was collected with help of sterilized spatula, knife etc. and were taken in poly bags and were brought to the laboratory. The soil samples were dried in air and sieved through 1 mm sieve. And were stored in plane container and were used for algal cultures.

### Pure culture of algae samples:

1gm of sieved soil samples were taken into sterilized beaker and 100 ml distilled water was added and 1 ml of the solution was streaked on directly agar plate in petri dishes containing De's modified Beneck's media. And the petri dishes were incubated under light for growth of algae. After the growth of algae single colony of algae was inoculated separately on another petri dish containing BG11 media simultaneously single colonies of different algae were inoculated in BG 11 liquid medium in 25 ml conical flask for mass culture at 20 to 25°C temperature for pure culturing (Plate 1). It is the preferred isolation method for many algae and most soil algae, not only for use but also axenic cultures can often be directly established without further treatment. The same conical flasks containing monoculture of algae were kept for further growth and were maintained by regular sub culturing for further studies.

## 3. Results and Discussion

### Pure Cultured Algal Samples

Following four pure cultured strains of algae are obtained. Identification was done by using morphological characters, thallus structure and colony characters considered as diagnostic features for identification and these morphological structures were identified by the standard Desikachary 1959 and some other books and various research articles.

### 1. *Gloecapsa* sp.:

Class: Cyanophyceae; Order: Chlorococcales; Family: Microsystaceae

The colonies of *Gloecapsa* start to grow generally after third day of inoculation. The pure culture of *Gloecapsa* is observed as heavy green mass generally settles down at the bottom of media. It is mucilaginous, compact, cells are spherical, having 3-4 μ diameter. Blue green in colour. The cells are having sheath very thick as thick as protoplast, very distinctly many times lamellated (Desikachary 1959).

### 2. *Phormidium* sp.:

Class: Cyanophyceae; Order: Oscillatoriales; Family: Oscillatoriaceae

The colonies of *Phormidium* start to grow slowly as compared to *Gloecapsa*. Pure culture of *Phormidium* in the flask appears in the form of bluish green lumps which generally float on the surface of media. It is more or less expanded, bright blue green. It is filamentous, variously entangled, having thin sheath, firm or diffident sometimes thick and more or less lamellate, violet coloured. Generally cells are shorter than broad, 1.5-2.71 μ long, rarely granulated at the cross wall, end cell rounded, calyptra absent (Desikachary 1959).

### 3. *Oscillatoria* sp.:

Class: Cyanophyceae; Order: Oscillatoriales; Family: Oscillatoriaceae

*Phormidium* starts to grow after fifth day of inoculation on the plates. 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 25-50 μ, 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 capitate without or with slightly thickened membrane (Desikachary 1959).



Figure a: Pure culture of *Gloeocapsa* in plate



Figure b: Pure culture of *Gloeocapsa* in flask

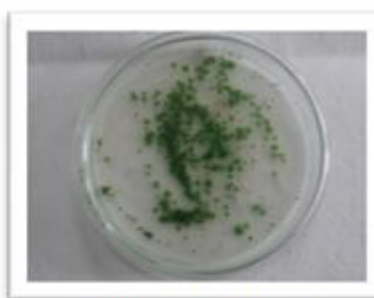


Figure c: Pure culture of *Phormidium* in plate



Figure d: Pure culture of *Phormidium* in flask

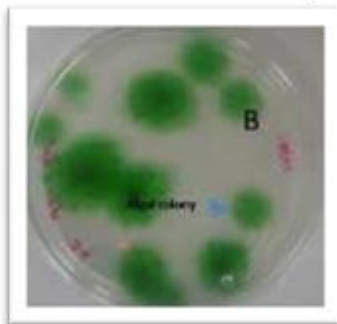


Figure e: Pure culture of *Oscillatoria* sp. in plate.



Figure f: Pure culture of *Oscillatoria* sp. in flask

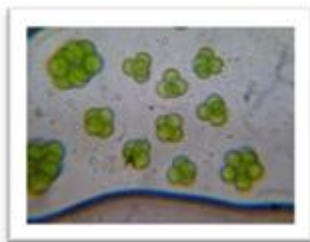


Figure f: *Gloeocapsa* sp.



Figure f: *Phormidium* sp.



Figure f: *Oscillatoria* sp.

**Elite strains of pure cultures of blue green algae from S.R.T.M.U. Nanded**

#### 4. Discussion

Microalgae have a great biological resource. They are used in various branches of science and technology. In present investigation three different strains of cyanobacteria mainly *Oscillatoria*, *Gloeocapsa*, *Phormidium* are pure cultured. Algal strains are isolated in axenic culture by the improved antibiotic method using streptomycin and fluconazole. Biodiversity of these blue green algae has been studied by different workers like Debnath (2009) from west Bengal studied diversity of *Phormidium*, *Oscillatoria*, *Gloeocapsa*, Biban and Singh (2011) from kurukshetra studied dominant cyanobacterial flora mainly *Oscillatoria*, Hazarika (2013) from Assam has done cyanophycean study and Makandar and Bhatnagar (2010) from Jodhpur studied most frequent genera of blue green algae. While very less work has been carried out on pure culturing of cyanobacteria. The cultures in the flask are formed in light green to dark blue green in colour some of them are formed in lumps while some are in separate. These pure cultures of algae have been kept to increase the biomass of particular species which will be used as biofertilizers.

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