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Research Article

Evaluation of Total Protein Production by soil Cyanobacteria in culture filtrate at various Incubations periods

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ABSTRACT

Cyanobacteria, also known as blue-green algae, are microscopic organisms that obtain their energy through photosynthesis, and are found in common and naturally occurring ecosystem sites like moist soil and water bodies. Cyanobacteria are in a range of shapes and sizes and can occur as single cells while others assemble into groups as colonies or filaments. Blue green algae produces many metabolites including amino acids, proteins, vitamins and plant growth regulators like auxins, gibberellins and abscisic acids. The present study has been undertaken to estimate the total protein in their culture filtrate during different incubation times using BG-11 broth and Pringsheim's broth. Protein was estimated from culture filtrate by standard protocols. The present study revealed that the amount of biomass, protein and IAA by different Cyanobacterial species were increased with the corresponding incubation time, and showed maximum concentration of 24 µg/ml, 14 µg/ml and 32.5 µg/ml in Pringsheim's broth after 30 days respectively.

Key Words: Cyanobacteria, Nitrogen fixation and Protein production

INTRODUCTION

Cyanobacteria belong to the group of organisms called prokaryotes, which also includes bacteria, and can be regarded as simple in terms of their cell structure. Cyanobacteria are present in almost all endolithic ecosystems; terrestrial and aquatic habitats such as oceans, fresh water bodies, moist soil, rocks, and even in rocks of the colder Antarctic. They can occur as planktonic cells or form phototrophic biofilms[14, 20]. They are characterised by the lack of a true cell nucleus and other membrane bound cell compartments such as mitochondria and chloroplasts. Cyanobacteria are also known as blue-green algae because of their prominent bluish green color, which is due to the phycocyanin pigment present in the cells. While, some species of cyanobacteria exhibit a reddish tone as they contain carotenoid and phycoerythrin pigments in their cells[18,19]. Cyanobacteria, are interesting group of phototrophic eubacteria whose diversity is unparalleled in the biological world, in terms of habitat, morphology or repertoire of metabolic activities. They proliferate in diverse types of ecosystems - ranging from the cold Tundra to the hot deserts, from surface waters of oceans to rhizosphere of plants and from barren lands/rock surfaces to polluted waters. Cyanobacterial inoculation has shown to enhance growth, root associated nitrogen fixation and yields of rice and other crops[12].

Proteins and amino acids are nitrogenous compounds and are also used as precursor molecule in protein synthesis. A lot of cellular and membrane components are made up of proteins. So organisms can synthesize amino acids as primary metabolites during their early growth phase. Cyanobacteria are considered to be the most diverse photosynthetic nitrogen fixing group, their thallus varies from unicellular to filamentous form. They are found in terrestrial habitat and in water. They fix atmospheric nitrogen in microaerophilic and aerobic conditions. In aerobic conditions, nitrogen fixation is carried out by

specialized cells found in filamentous form called heterocyst. Heterocystous cyanobacteria showed a marked effect on the cell density and diversity by the use of urea in soil fertilization[11]. They fix nitrogen assimilated in the form of amino acids and proteins. These nitrogenous compounds increase the overall soil fertility[8].

Cyanobacteria characteristically liberate substantial quantities of extracellular nitrogenous compounds into the culture medium like amino acids, proteins, vitamins. These same compounds are also available in soil after growth of Cyanobacteria, thus boosting soil fertility. Hence they function as effective biofertilizer. They are also responsible for the production of plant growth regulators like auxins, gibberellins and abscisic acid[3]. Cyanobacteria are responsible for producing combined nitrogen in soluble form by nitrogen fixing BGA.

The objective of the present work is to isolate cyanobacteria, estimate their total protein under different incubation times using BG-11 broth and Pringsheim's broth evaluated via standard protocols[6].

MATERIAL AND METHODS

Sample Collection

Soil samples were collected from cotton, rice and sugarcane fields around Surat and Navsari districts of Gujarat, India. The average pH of the soil is around 6.2. Samples were first screened to remove plant debris and rock particles.

Isolation of Blue Green Algae[17]:

1. 10 gram soil was serially diluted via a standard dilution technique using Erlenmeyer conical flask having 95 ml 0.85% saline water to attain10^{-1} dilution.
2. 10 ml sample was transferred from 10^{-1} to next conical flask containing 90 ml saline water to further attain 10^{-2} dilution.
3. 10 ml sample was transferred from dilution 10^{-2} to conical flasks having100 ml BG-11 broth (nitrogen free).
4. The flasks were incubated at Room Temperature (R.T.) in a continuous illuminated chamber for 21 days.
5. Microscopic observation was done by spreading cyanobacterial culture on glass slide covered with a cover slip.
6. Low and high power objective lens of compound light microscope were used for observation.
7. Mixed cultures were again streaked on BG-11 agar plate and incubated at Room Temperature in a continuous light (illuminated) chamber for 21 days[7].
8. Microscopic slides were then prepared for final observations and analysis.

Identification of Blue Green Algae[1, 2, 9]:

1. Microscopic observations were carried out by spreading isolated culture on glass slide using forceps.
2. Culture was covered with glass cover-slips and observed under low (10 X) and high power (45 X) objective lens.
3. Pure forms of cyanobacteria were identified on the basis of morphological characteristics following the standard procedure mentioned in Bergey's Manual of Systematic Bacteriology, Vol 1[4, 5].
4. The cultures thus identified belong to Genus *Cyanothece, Cyanobium and Nostoc.*

Growth and Maintenance[10, 13]:

1. Identified cultures were grown and maintained in chemically defined nitrogen free BG – 11 medium at Room Temperature in a continuously illuminated chamber.
2. pH of the medium was strictly maintained at 7.5 for the optimal growth of cultures.
3. After 14 days of incubation, the cultures were subjected to streaking on agar based BG – 11 medium for obtaining discrete colonies.
4. The purity of cultures was examined by microscopic observation from the plates.

Protein Production

Culture was transferred to separate conical flasks containing 100 ml BG-11 broth and Pringsheim's broth, followed by incubation at 30 ºC for 10,15, 20, 25 and 30 days in continuously illuminated chamber. Culture filtrate was obtained by filtration using Whatman No. 1 filter paper or by centrifugation.

Estimation of Total Protein by Lowry Method

Lowry reagents[15] were mixed with 0.5 ml culture filtrate and incubated at 37 ºC for 30 minutes at Room Temperature. Color density was recorded at 750 nm using spectrophotometer and compared with standard curve of protein (BSA, 200 µg/ml).

RESULTS & DISCUSSION

Culture filtrates were obtained via vacuum filtration using membrane filter paper and total protein was estimated by Folin Lowry's method. The results obtained with regard to protein production by cyanobacteria are presented in Table-1 and Figure-1.

From Table-1 and Figure-1, it can be concluded that 24 µg/ml, 32.5 µg/ml and 14 µg/ml protein were produced by culture 1, 2 and 3 respectively within 15 days incubation time respectively.

Figure- 1 indicates that the amount of protein has increased up to 15 days incubation time, and then decreased due to the protein being utilized for their building blocks.

All the three species produced a maximum amount of protein up to 15 days.

On evaluation, it was observed that the amount of biomass and extracellular protein was increased with the increase in incubation time up to 15 days. It was found to be produced in early growth phase and concentration increased very rapidly. Protein is a nitrogenous compound and is available in culture filtrate of all the three species viz. *Cyanothece* species, *Cyanobium* species, *Nostoc* species[7,16].

CONCLUSION

For microbial proteins to be measured accurately, the cells must be pretreated to fully release the intracellular proteins. Pretreatments typically involve disrupting the cells by physical or chemical means Hydrolytic enzymes and chemicals such as sodium dodecyl sulfate (SDS) can be used to lyse the cell walls but not all cells are equally susceptible to enzymes and chemicals. Therefore physical treatment is preferable**.** In our study of estimation it was found that amount of extracellular proteins as a primary metabolite were increased during log phase by cultures 1, 2 and 3 with increasing incubation time on 15th day. Protein production was reduced in late growth phase and slowly concentration was decreased. Cyanobacteria liberates growth promoting substances like amino acids and other primary metabolites in their culture filtrate, which can be used as source of nitrogen for plant growth. These amino acids help to increase the soil fertility.

Table 1
Estimation of Total Protein in Culture Filtrates of Cyanobacterial species

S. No.	Incubation Days	Total Protein (µg/ml)		
		Culture: 1	Culture: 2	Culture: 3
01	05	3.5	2.5	2.0
02	10	21.5	31.0	10.0
03	15	24.0	32.5	14.0
04	20	14.0	9.5	7.0
05	25	6.5	6.5	5.0
05	30	3.5	5.0	5.0

Note: Cultures: 1 Cyanothece species; 2 Cyanobium species; 3 Nostoc species

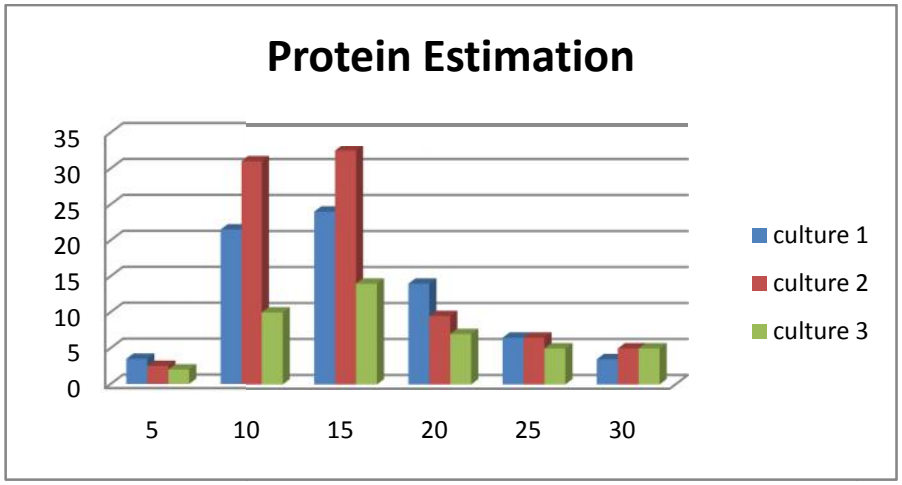

Figure 1
Total Protein Estimation in Culture Filtrates of Cyanobacterial species

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