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Research Article

Study of Soil Cyanobacteria to Evaluate Metabolite Production during various incubations in their Culture Filtrate

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Abstract: Cyanobacteria are a remarkable group of photosynthetic prokaryotes. It survives in wide range habitat in soil and also morphologically various from unicellular to filamentous thalli. Blue green algae produced many metabolites including amino acids, proteins, vitamins and plant growth regulators i.e. auxins, gibberellins and abscisic acids. The present study is to estimate biomass, total protein and plant growth hormone (Indole-3-acetic acid) in their culture filtrate during various incubation times using BG-11 broth. Protein and Indole-3-acetic acid was estimated from culture filtrate by standard protocols. By observing it was found that amount of biomass, protein and IAA was increased with incubation time and shows maximum concentration as 300 mg, 750 µg/ml and 4.0 mg/L after 30 days respectively by *Nostoc* spp.
Keywords: Cyanobacteria, Biomass, Protein, Indole-3-acetic acid, Incubation time.

INTRODUCTION

Blue green algae are the most diverse and dominant photosynthetic prokaryotes found in soil that secrets carbon and nitrogen compounds in soil [10]. The plant growth regulators e.g. gibberellin, auxin, cytokinin, ethylene and abscisic acid have been detected in cyanobacteria [14, 15]. Cyanobacterial biomass extracts of *Plectonema* spp. stimulated somatic embryogenesis and development in sandalwood, *Santalum album* [2].

Cyanobacterial strains forming mucilaginous colonies which help to dominance of this organism in soil especially *Nostoc* spp. [15]. Comparative biomass production and nitrogen fixation has been examined in large number of cyanobacterial strains including members of Rivulariaceae such as *Gloeotrichia* and *Calothrix* and reported that *Gloeotrichia* spp. exhibited higher dry weight and *Calothrix* higher nitrogen fixation [6, 19].

Cyanobacteria characteristically liberate substantial quantities of extracellular nitrogenous compounds into the medium and also produced combined nitrogen in soluble form by nitrogen fixing BGA [21, 22]. They add organic matter and secrete growth promoting substances like vitamins, auxin and amino acids [17, 20].

The cyanobacteria are a remarkable group of prokaryotes which can survive independently and also in association with other organisms such as lichen, liverwort and mosses [3]. They secrets commonly plant growth regulators (auxins, gibberellins, and ethylene), amino acids, protein and vitamins. Indole-3-acetic acid is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including cyanobacteria [8].

Libration of extra-metabolites has been found maximum during the stationary phase of growth. The growth regulators reported in rice fields are gibberellins, auxin, cytokinin, ethylene, and abscisic acid by addition of cyanobacterial biofertilizer [14, 18] and their extracts promote overall plant growth [11, 12, 16]. The objectives of the present work is to produced cyanobacteria biomass, total protein and Indole-3-acetic acid during different incubation time using BG-11 broth and evaluated via standard protocols.

MATERIAL AND METHODS

Sample collection

Six to eight soil samples were collected from agricultural fields around Ratlam district, M.P., India. Composite sample was obtained via mixed all samples together and grinded using mortar and pestle the soil sample was clean to remove plant debris and rock particles.

Isolation of blue green algae

Weigh 10 g soil and serially diluted using a standard dilution technique [1] and BG-11 broth with nitrogen [7]. The soil sample was dissolve in Erlenmeyer conical flask having 95 ml 0.85% saline water and mixed well, mark as 10^{-1} dilution. 10 ml sample was transferred from 10^{-1} dilution to next conical flask containing 90 ml saline water, marks as 10^{-2} dilution. One milliliter sample was transferred to three tubes having 10 ml BG-11 broth and tubes were incubated at 30ºC in a continuously illuminated chamber, 4000 lux, for 3 weeks [7].

Identification of blue green algae

Microscopic observation was done by spreading isolated culture on glass slide using forceps

and needles. Culture was covered with glass cover slips and observed under low (10X) and high power (40X) objective lens of compound light microscope. Pure forms of cyanobacteria were identified on the basis of morphological characteristics mentioned in Bergey's Manual of Determinative Bacteriology and Bergey's Manual of Systematic Bacteriology, 2nd ed. Vol. 1 [4, 5].

Biomass Production

Pure culture of *Nostoc* spp. was inoculated in conical flask containing 100 ml BG-11 broth and incubated at 30ºC for 10, 15, 20, 25 and 30 days in continuously illuminated chamber, 4000 lux. Biomass was obtained by filtration using Whatman No. 1 filter paper and dry in hot air oven at 50ºC for 2 hrs to remove extra moisture then weigh total biomass [7].

Protein production

Nostoc spp. was transferred to conical flask have 100 ml BG-11 broth and incubated at 30ºC for 10, 15, 20, 25 and 30 days in continuously illuminated chamber. Culture filtrate was obtained by filtration using Whatman No. 1 filter paper.

Estimation of total protein by Lowry method

Lowry reagents [13] were mix with 0.2 ml culture filtrate and incubated at 37ºC for 20 min. in water bath. Color density was recorded at 660 nm using spectrophotometer and compared with standard curve of protein (BSA, 1.0mg/ml).

Indole-3-acetic acid (IAA) production

Selected cyanobacterial isolate i.e. *Nostoc* spp. was transferred to Erlenmeyer conical flask containing 100 ml BG-11 broth. The conical flask was incubated at 30ºC for 10, 15, 20, 25 and 30 days in continuous light exposure and culture filter was obtained by filtration using Whatman No.1 filter paper then used for extraction.

Extraction

Firstly set pH of culture filtrate at 2.8 with HCl then IAA was extracted three times with three volumes of ethyl acetate using glass separating funnel and mixed. The extract was evaporated at 37ºC using water bath and adjusted pH 7.0 with NaOH.

Estimation of IAA by Salkowski method

3 ml extract was taken in test tube and added 2 ml salkowski reagent [9]. The test tube was incubated at room temperature for 30min. and the color density was recorded at 530 nm using spectrophotometer. The optical density (OD) of sample was compared with standard curve of IAA (500 µg/ml).

RESULTS AND DISCUSSION

The biomass and metabolite estimation of various incubation extract was evaluated using standard protocols and have found following estimations given in Table-1 to 3.

Results obtained to evaluate total biomass are shown in Table-1 and Fig. - 1 that biomass was increased as incubation period increased. Further Table-1 indicates that minimum biomass (40 mg) produced within 10 days; metabolites are utilized by *Nostoc* spp. then cyanobacteria produced more biomass (120 mg) within 15 days and then growth of *Nostoc* spp. was slowly increased. After 25 days growth was enhanced due to available of more growth regulator in medium and produced 300 mg biomass, recorded after 30 days incubation time [7].

Table-2 and Fig.-2 reveals that 195 and 750 µg/ml protein was produced by *Nostoc* spp. within 20 and 30 days incubation time respectively. Fig.- 2 indicates that amount of protein was increased up to 15 days incubation time then decreased due to utilization for their building blocks. The concentration of protein was increased again and reaches maximum as 361 and 750 µg/ml within 25 and 30 days respectively [7].

Results obtained from Table - 3 and Fig. - 3 that IAA produced in culture filtrate of *Nostoc* spp. after 25 and 30 days incubation time that is 1.13 and 4.0 mg/L respectively. Indole-3-acetic acid is a plant growth regulator and its very minute quantity required for their growth. *Nostoc* spp. multiplied in soil and synthesized then secrets extracellular IAA during stationary condition which available in soil for plant growth [11, 12, 16].

Table– 1: Estimation of total biomass of *Nostoc* spp.

S. No.	Incubation days	Biomass (mg)
1	10	40
2	15	120
3	20	160
4	25	180
5	30	300

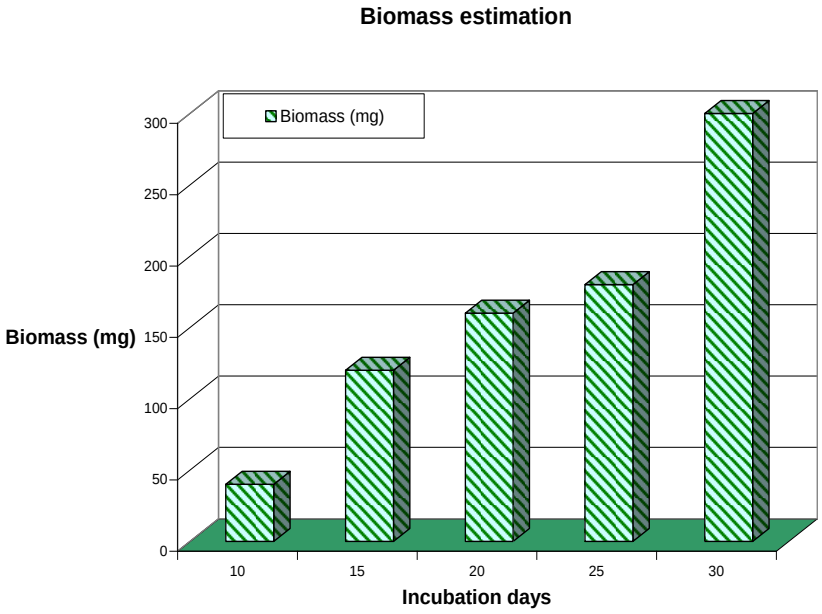

Figure-1: Total biomass estimation of *Nostoc* spp. during different incubation time.

Table-2 Estimation of total protein in culture filtrates of *Nostoc* spp.

S. No.	Incubation days	Total Protein (µg/ml)
1	10	466
2	15	581
3	20	195
4	25	361
5	30	750

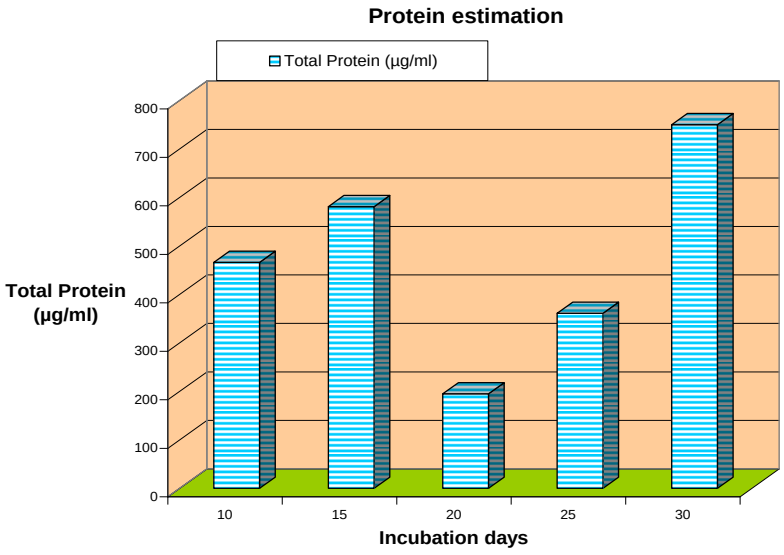

Figure- 2: Total protein estimation in culture filtrates of *Nostoc* spp.

Table-3 Estimation of IAA in culture filtrates of *Nostoc* spp.

S. No.	Incubation days	IAA (mg/L)
1	10	0.0
2	15	0.0
3	20	0.0
4	25	1.13
5	30	4.00

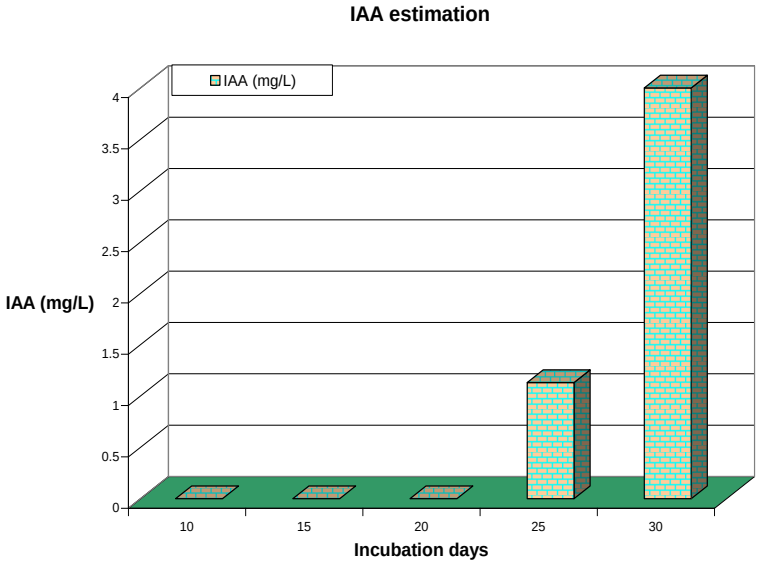

Figure- 3: Indole-3-acetic acid estimation in culture filtrates of *Nostoc* spp.

CONCLUSION

By evaluating it was found that amount of biomass and extracellular protein was increased with increasing incubation time but Indole-3-acetic acid was detected after 25 days, it produced in late growth phase and slowly concentration was increased. Both protein and IAA is nitrogenous compound and available in culture filtrate of *Nostoc* spp. Similarly also available in soil after growth of cyanobacteria and improves their fertility as biofertilizer.

REFERENCES

1. Allen, MM; Methods for Cyanophyceal. Stein, J.R. (ed). Handbook of phycological methods, culture methods and growth measurements, Cambridge Univ. Press, 1973, 127-138.
2. Bapat VA, Iyer, RK and Rao, PS; Effect of cyanobacterial extract on somatic embryogenesis in tissue cultures of sandalwood. J. Medicinal and Aromatic Plant Sci., 1996, 18: 10-14.
3. Belnap J, Budel, and Lange, OL; Biological soil crusts: characteristics and distribution. In: J. Belnap and O. Lange (ed.). Biological Soil Crusts: Structure, Management and Function. Ecological Studies, 2003, 150: 3-30. Springer-Verlag, Berlin.
4. Boone, DR and Castenholz, RW; Bergey's manual of systematic bacteriology, 2nd ed. Vol.1 Springer, USA, 2001.
5. Buchanan, RE and Gibbons, NE; Bergey's manual of Determinative bacteriology. Williams and Wilkins, Baltimore, Maryland, 1994.
6. Dikshit, G and Tiwari, GL; Effect of pesticides on certain nitrogen fixing cyanobacteria. Proc. Nat. Symp. Cyanobacterial nitrogen fixation, 1990, 81-86.
7. Dubey, V; Studies on physiology and ecology of cyanobacteria from grasslands of Ujjain and to evaluate their potentiality as biofertilizer. PhD thesis, IEMPS, Vikram Uni. Ujjain, 2007.
8. Frankenberger WT and Brunner, W; Methods of detection of auxin (indole-3-acetic acid) in soils by high performance liquid chromatography. Soil Science of American Journal, 1983, 47: 237-241.
9. Gordon SA and Weber, RP; Colorimetric estimation of Indoleacetic acid. Plant Physiology, 1951, 26: 192-195.
10. Irisarri P, Gonnet, S and Monza, J; Cyanobacteria in Uruguayan rice fields: diversity, nitrogen fixing ability and tolerance to herbicides and combined nitrogen, 2001.

11. Javaid, A; Effects of biofertilizers combined with different soil amendments on potted rice plants chilean Journal Of Agricultural Research, 2011, 71(1):157-163.
12. Kotnala, S; Blue green algae as a potential source of growth promoter in common Indian rice, *oryza sativa* (linn.) Geobios, 2003, 30: 70-73.
13. Lowry OH, Rosebrough, NJ, Larr, AL and Randall, RJ; Protein measurment with Folin-phenol reagent. J. Biol. Chem., 1951, 193: 265-275.
14. Ordog V and Pulz, O; Diurnal changes of cytokinin like activity in a strain of Arthronema africanum, determined by bioassays. Algol. Stud., 1996, 82: 57-67.
15. Roger PA, Santiago-Ardales, S, Reddy, PM and Watanabe, I; The abundance of heterocystous blue-green algae in rice fields. Biol Fertil Soils, 1987, 4: 98–105.
16. Selvakumar G, Sundararaman, M; Influence of cyanobacteria on improving alkaline soil fertility, growth and yield of paddy. J.Sci.Trans.Environ.Technov., 2009, 2(3): 135-138.
17. Shen-Rui Z and Shen, RZ; A broad spectrum biopesticide type biofertilizer anaerobic fermentation effluent and plant adverse resistance. Acta. Agr. Shanghai, 1997, 13(2): 89-96.
18. Stirk WA, Ordog, V and Staden, J; Identification for the cytokinin isopentenyladenine in a strain of *Arthronema africanum*. J. Physiol., 1996, 35: 89-92.
19. Tripathi IC, Ayyappan, S and Adhikary SP; Isolation and growth of local heterocystous cyanobacteria for using as biofertilizer. Proc. Nat. Symp. Cyanobacterial Nitrogen Fixation, (ed.) Kaushik BD, Indian Agricultural Research Institute, 1990, 367-370.
20. Varga L, Szigeti, J and Ordog, V; Effect of Spirulina platensis biomass enriched with trace elements on combinations of starter culture Isolates employed in the dairy industry. Milchwissen-Schaft, 1999, 54(5): 247-248.
21. Venkataraman, GS; Algal biofertilizer and rice cultivation. Today and Tomorrows printers and publishers, New Delhi, 1972.
22. Vorontsova GV, Romansova, NI, Postnova, TI, Selykh, IO and Gusev, MV; Bio-stimulating effect of cyanobacteria and ways to increase it. I. Use of nutrients – super products of amino acids. Moscow University Biological Sciences Bulletin, 1988, 43: 14-19.