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ESTIMATION OF NITROGENASE ACTIVITY IN SOME SELECTED STRAINS OF BLUE-GREEN ALGAE

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ABSTRACT

Blue green algae are mostly concerned in their utilization as nitrogen fixing organism, it is on these ground scientists around the globe are interested in biofertilizer technology. The present study deals with the estimation of nitrogenase activity in 24 selected strains of blue green algae. Nitrogenase activity of different strains was observed in nitrogen deficient medium. All the strains showed the range of nitrogenase activity in between 1.92n mole C_2H_4 /µg chl-a/h to 7.82n mole C2H4/µg Chl-a/h.

Keywords: Blue green algae, Nitrogen, Nitrogenase activity.

1. INTRODUCTION

Nitrogen being the most constituents of proteins, enzymes, DNA and many other biomolecules, is an essential requirement of all the plants and animals, and they depend on synthetic nitrogen source like nitrate, ammonia and urea etc. However biological nitrogen fixation is performed by only prokaryotic organism like "bacteria and blue green algae". Not all bacteria not all blue green algae can fix nitrogen. Blue green algae are the largest contributers to the process of biological nitrogen fixation the second most biological process on this planet [1]. The conversion of elemental diatomic nitrogen to ammonia is known as nitrogen fixation. This is generally achieved industrially at very high temperature and pressure in presence of catalyst. The same process when carried out by certain prokaryotic micro-organisms at ambient temperature and normal atmospheric pressure in the presence of enzyme nitrogenase is called Biological nitrogen fixation.

The roll of nitrogen fixating blue green algae in the maintenance of the fertility of rice field has been well substantiated and documented [2-11]. Several researchers [12-14] undertook an extensive survey of blue green algae of paddy field of several states during 1962-1967 and perform a series of pot experiment to evaluate their roll in nitrogen fixation. The physiological characterization of blue green algae have been studied by numerous workers and most of workers and most of the work have been reviewed by different researchers [1,15-28]. The growth and development of plants depends upon nitrogen, therefore, Nitrogen is one of the most important element in plants. It was considered for a longer time that certain blue green algae could fix atmospheric nitrogen but for a long time it was considered that only heterocystous members of blue green algae were reported as nitrogen fixers where heterocysts have been found to be the site of nitrogen fixation [25,27]. The heterocysts were found in the absence of nitrogenous substances in their natural medium [29-32]. But later on it was proved that certain non heterocystous blue green algae could also fix atmospheric nitrogen under anaerobic conditions i.e. *Phormedium favealane* [33] *Oscillatoria* spp. [34-36], *Lyngbya aesturaii* [37]; *Plactonema boryanum* [38].

2. EXPERIMENTAL

The determination of nitrogenase activity has been precisely done to compare the physiological characterization of selected strains. Inoculums have always been taken from exponential growth phase of cultures (7-15 days grown cultures).

Estimation of nitrogenase activity: The most commonly used method for estimating the rate of N_2 fixation is the C_2H_2 reduction technique [39]. The product of C_2H_2 reduction is C_2H_2, which is easily separated by gas-liquid chromatography. It was measured by using nitrogen as the carrier [40]. The acetelyne reduction assay (ARA) was done through gas chromatograph (Amil Nucon 5700) attached with Flame Ionization Dector (FID). Nitrogenase activity was observed by *in-situ* acetylene reduction technique.

Preparation of acetylene: Acetylene was produced by calcium carbide(CaC_2) with addition of water and stored into a reservoir after passing through an acidic $CuSO_4$ solution to make free from phosphine and other impurities.

$$CaC_2 + 2H_2O \longrightarrow Ca(OH)_2 + C_2H_2$$

Procedure: The acetylene reaction assay involved withdrawing 5 mL of a 10 mL algal sample and placing it in a reaction vial. Air phase was maintained and then 10%v/v of the air phase was replaced with acetylene by syringe injecting inside the reaction vials. After that these vials were incubated for 2 h under 5000lux light intensity and 300 ± 2 ºC. After incubation period, the algal samples were inactivated by injecting 0.1 mL 50% TCA (trichloroacetic acid). Then known volume (0.5 mL) of gas phase of each vial was withdrawn as a sample and injected into the injector port of the gas chromatograph. The reduced quality of C_2H_2 and C_2H_4 was measured on PC, hooked by interphase card.

Appropriate standard was made from the pure ethylene gas (103 vpm ethylene in Argon) and find out value of K. The relative unit was taken peak area/unit (in UV/S). Nitrogenase activity was expressed as "n mole" C_2H_2 produced per µg chlorophyll per hour (n mole C_2H_2/µg chl./h) and it is also presented as n mole C_2H_2/vial/hour.

Calculation:

$$\text{Nitrogenise activity} = \text{N mole } C_2H_4/\mu g \text{ Chl-a/h} = (P^1 \times V)/(K \times P \times t \times m)$$

where, P^1 = Peak area or peak height of sample; V = volume of gas (air) phase; K = Constant volume of standard ethylene; P = Volume of injected sample; T = Incubation time of sample (in h); M = Total chlorophyll-a in µg

3. RESULTS & DISCUSSION

In present study, total 24 strains were taken for physiological characterization, out of which 1 strain of Cylindrospermum, 5 strain of Nostoc, 4 strain of Anabaena, 1 strain of Aulosira, 1 strain of Microchaete, 1 strain of Tolypothrix, 1 strain of Camptylonemopsis, 2 strain of Scytonema, 4 strain of Calothrix, 1 strain of Gloeotrichia, 1 strain of Chlorogloeopsis, 1 strain of Westiellopsis and 1 strain of Hapalosiphon has been taken for estimation of nitrogenase activity.

Estimation of Nitrogenase activity: Nitrogenise activity (n mol C_2H_2/µg chl./h) of different strains was observed in nitrogen deficient medium after 15 days of time interval. The results indicated that all the strain showed the range of Nitrogenase activity in between 1.92n mole C_2H_2/µg chl./h / µg chl-a/h to 7.82n mole C_2H_2/µg chl./h. Best nitrogenise activity was observed in Tolypothrix-46 (7.82 n mole C_2H_2/µg chl./h) while lead in Calothrix-44 (1.92n mole C_2H_2/µg chl./h).

In regard to nitrogenise activity the following trend was observed: Tolypothrix-46 > Anabaena-24 > Aulosira-52 > Anabaena-49 > Hapalosiphon-19 > Scytonema-43 > Anabaena-32; Microchaete-11 > Calothrix-50 > Nostoc-28 > Camptylonemopsis-39 > Calothrix-25 > Cylindrospermum-21 > Westiellopsis-33 > Nostoc-35 > Nostoc-51 > Nostoc-15 > Chlorogleopsis-40 > Gloeotrichia-47 > Scytonema-12 > Anabaena-7 > Calothrix-31 > Calothrix-44 (Table-1 & Fig. 1).

Table 1: Table-1: Analysis of Nitrogenase activity in nitrogen free BG-11 medium on 15 day of growth

S.No.	STRAINS	Nitrogenase Activity n mol (C_2H_4/μg chl-a/h)
1.	Cylindrospermum-21	3.57
2.	Nostoc-35	3.19
3.	Nostoc-51	2.97
4.	Nostoc-28	3.92
5.	Nostoc-42	3.87
6.	Nostoc-15	2.84
7.	Anabaena-7	2.15
8.	Anabaena-24	5.98
9.	Anabaena-49	4.92
10.	Anabaena-32	4.22
11.	Aulosira-52	5.68
12.	Microchaete-11	4.21
13.	Tolypothrix-46	7.82
14.	Camptylonemopsis-39	3.87
15.	Scytonema-12	2.50
16.	Scytonema-43	4.79
17.	Calothrix-25	3.82
18.	Calothrix-31	2.05
19.	Calothrix-44	1.92
20.	Calothrix-50	3.94
21.	Gloeotrichia-47	2.68
23.	Chlorogleopsis-40	2.84
24.	Westiellopsis-33	3.56
25.	Hapalosiphon-19	4.87

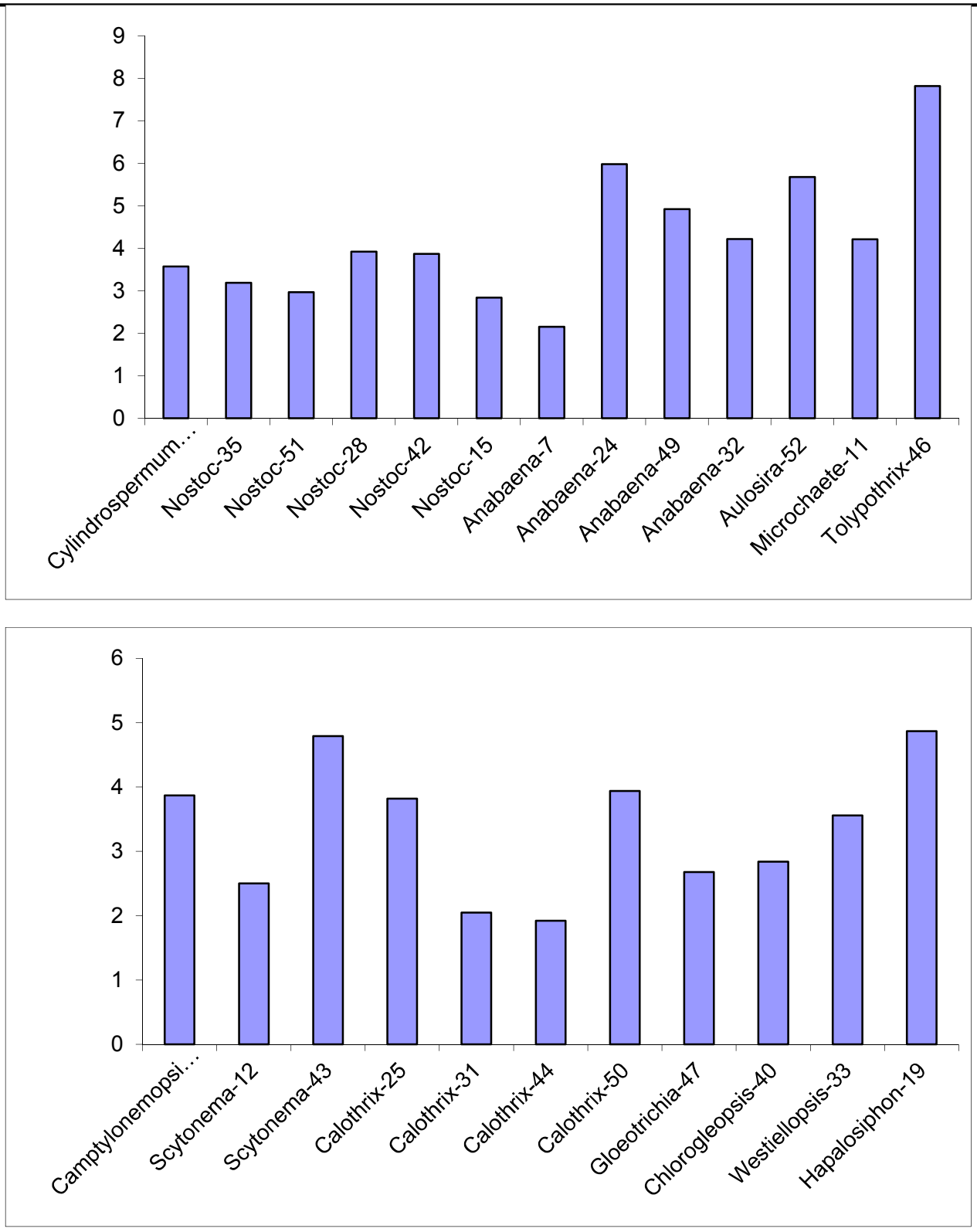
9
8
7
6
5
4
3
2
1
0
Cylindrospermum…
Nostoc-35
Nostoc-51
Nostoc-28
Nostoc-42
Nostoc-15
Anabaena-7
Anabaena-24
Anabaena-49
Anabaena-32
Aulosira-52
Microchaete-11
Tolypothrix-46
6
5
4
3
2
1
0
Camptylonemopsi…
Scytonema-12
Scytonema-43
Calothrix-25
Calothrix-31
Calothrix-44
Calothrix-50
Gloeotrichia-47
Chlorogleopsis-40
Westiellopsis-33
Hapalosiphon-19

Fig. 1

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