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Evaluation of Phycobilin Pigment Content in *Nostoc muscoruma* at different pH

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ABSTRACT
Nostoc muscorum is a free-living microorganism is distributed over a large area of the globe in many varying habitats, both aquatic and terrestrial. It is known to form symbiotic relationships with terrestrial plants and is common in desert crusts and benthic communities of water-logged paddy fields. The ideal environment for N. muscorum is one with pH in the range of 7.0 to 8.5. It was cultured in medium with pH ranging from 7 to 11 and observed and evaluated for morphological and physiological characteristics like heterocyst frequency, and phycobilin pigment content. The result reveals that alkaline pH ranging from pH 8 to pH 10 is suitable for the growth and physiological activities of N. muscorum.
Keywords: *pH, Phycobilin, Heterocyst, N. muscorum*

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INTRODUCTION

Cyanobacteria can be found in almost every terrestrial and aquatic habitat oceans, fresh water, damp soil, temporarily moistened rocks in deserts, bare rock and soil, and even Antarctic rocks. They can occur as planktonic cells or form phototrophic biofilms. They are found in almost every endolithic ecosystem [1]. A few are endosymbionts in lichens, plants, various protists, or sponges and provide energy for the host [2]. Cyanobacteria fulfill vital ecological functions in the world's oceans, being important contributors to global carbon and nitrogen budgets.Cyanobacteria also form symbiotic association with animals and plants. Symbiotic relations exist with, for example, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms [9]. *Nostoc muscorum* is a free-living microorganism is distributed over a large area of the globe in many varying habitats, both aquatic and terrestrial. Its ability to populate such a diverse range of conditions is due the cellular development of metabolic adaptations to harsh conditions, such as desiccation-tolerance, salt-tolerance, and nitrogen-fixation processes. As cyanobacteria are phototrophic, performing photosynthesis in their environments requiring CO_2 for growth and also fixing atmospheric nitrogen. The ideal environment for *N. muscorum* is one with pH in the range of 7.0 to 8.5, with a lower pH limit of 5.7 [3]. It grows best when light intensity is less than that of direct sunlight, [4]. *N. muscorum* has heterocyst, which are specialized nitrogen-fixation cells. Heterocyst, (5-10% of cells) appear when it is transferred to nitrogen free media. Appearance of heterocyst is concurrent with an increase in nitrogenase activity, which reduces N_2 to NH_3. It fixes nitrogen that is important in symbiotic relationships with fungi, liverworts, hornworts, mosses, cycads [7].

MATERIAL AND METHODS

Establishment of culture:
The algal culture was established in liquid Fogg's medium (0.2gm $MgSO_4.7H_2O$, 0.2gm K_2HPO_4, 0.1gm $CaCl_2.H_2O$, 5 ml FeEDTA (dissolve 0.745gm Na_2EDTA in hot water & add 0.557gm $FeSO_4$, boil to dissolve completely, final volume to 100ml), 1ml/litre, micronutrients (286 mg H_3BO_3,18 mg $MnCl_2.4H_2O$, 22 mg $ZnSO_4.7H_2O$, 39 mg $Na_2MoO_4.2H_2O$, 8mg $CuSO_4$, 4 mg $CoCl_2$), pH 7.5, final volume to 1 liter.)

5g of algal inoculums was added to each culture bottle in aseptic conditions. The culture was maintained in the diffused sun light for the period 30 days to obtain sufficient growth. Alga was sub-cultured in open culture system using culture trays. 1 liter of Fogg's medium was added per tray. The culture trays were divided into eight sets of different pH from pH 4 to pH 11 each containing 15 trays. This culture was allow to grow for 1 week & then used for further analysis.

Measurement of pH:

The initial pH was measured after 1 week of subculture. The change in pH was observed. The Fogg's medium of pH 7.5 was added to each set and then pH was measured on each day for successive days.

Study of Morphology:

The morphological observation with respect to growth and thallus morphology was done for each set of pH and the observations were recorded.

Determination of Heterocyst Frequency:

The slides were prepared for each set of pH and observed under compound microscope (45x magnification). Number of heterocyst were counted from different 5 fields on slide and mean was calculated and frequency was determined for each set.

Estimation of Phycobilin Pigment Content:

The total phycobilin pigment content was determined by protocol given by Evans Methods. 0.5g of algal culture was crushed in the 5 ml of 0.1M phosphate buffer, pH 6.8(25.5 ml 0.1M NaH2PO4, 24.5 ml Na_2HPO_4, final volume 100 ml, pH 6.8).with acid washed sand using chilled mortar& pestle. This homogenate was transferred into centrifuge tube & spin at 5000 rpm for 10 min. Final volume of supernatant was set to 25 ml using 0.1M phosphate buffer, pH 6.8. The resultant solution was used as sample for further investigation. The absorbance was measured using spectrophotometer at wavelength 455nm, 564nm, 592nm, 618nm, 645nm and 650nm; for c-Phycocyanin (c-PC), c-Phycoerythrin (c-PE) and Allophycocyanin (APC) Amount of c-PC, c-PE and APC was determined by using formulae as given in protocol.

RESULTS AND DISCUSSION

The morphological study reveals that growth of *Nostoc muscorum* at acidic pH from pH 4-pH 5 was less as compared to neutral and alkaline pH ranging from pH 8-pH 11. The color of thalii observed was from yellowish green to dark green with increase in pH. (Figure No.1 &2, Table No. 1).

Figure No. 1: Culture of *N. muscorum*

Figure No. 2: Sub-culture of *N. muscorum*

Table No. 1: Morphology

pH	Morphological Features
4	Less growth, yellowish green
5	Less growth, yellowish green
6	Moderate growth, thick, dark green
7	Moderate growth, thick, light green
8	High growth, pale yellowish green
9	High growth, thick, greenish yellow
10	Highest growth rate, dark green
11	Highest growth rate, dark green

Table No. 2: Heterocyst Frequency

pH	Mean Heterocyst	Frequency %
4	3.4	68
5	3.8	76
6	4.8	96
7	4.4	88
8	4.4	88
9	4.6	92
10	4.2	84
11	5.8	116

Table No. 3: c-Phycocyanin, Allophycocyanin and c-Phycoerythrin Content

pH	c-PC (mg/L)	APC (mg/L)	c-PE (mg/L)
4	7.642	1.4097	8.8014
5	2.9584	5.4911	11.8714
6	9.228	0.2518	7.0363
7	2.496	3.031	6.5688
8	6.0744	0.07788	9.3278
9	2.812	1.9356	5.8121
10	2.994	2.8741	6.4344
11	2.04	2.3448	3.9686

According to earlier studies, it is found that cyanobacteria cultured at three different pH conditions as, 7.0, 9.5, 10.5. The higher value of growth rate, cell yield observed in pH 10.5. The low pH limits the growth in terms of no. of cells & biomass. (Figure No.2, Table No. 1)

The heterocyst frequency was found to be increased with increase in pH 4 to pH 11. This concludes that maximum no. of heterocyst are formed in alkaline pH than the acidic pH. (Table No. 3)

Among the studied phycobilin pigments content was observed at acidic pH 5 and minimum amount in alkaline pH 8. The same trend was observed for the carotenoid content also. (Table No. 4)

CONCLUSION

Effect of pH on morphological and physiological characteristics of *Nostoc muscorum*: The data on growth, number of heterocyst, chlorophyll pigment, carotenoid that the organism has ability to create and maintain the favorable growth conditions for its growth by temporary changes in metabolism.

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