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European Journal of Experimental Biology, 2016, 6(4):23-27

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Influence of light quality on extracellular ammonium excretion by cyanobacteria isolated from Loktak Lake, the only largest freshwater lake in the North-Eastern region of India

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ABSTRACT

Cyanobacteria experience changes in light quantity and light quality in their natural habitat. In response to changes in light quality, they re-distribute excitation energy and adjust photosystem stoichiometry to maximize the utilization of available light energy. In the present study, six (06) cyanobacterial strains namely, Nostoc sp. BTA60, Nostoc sp. BTA61, Nostoc commune BTA67, Calothrix sp. BTA73, Nostoc sp. BTA80 and Nostoc muscorum BTA950 were cultured under different qualities of light modes (white, blue, green and red) to ascertain on the excretion of extracellular ammonium. Among the four light qualities used, white light was found to be most effective followed by red, green and blue light in all the strains tested. Nostoc muscorum BTA950 released maximum extracellular ammonium (236.98 µgml^{-1}) in white light followed by red light (231.77 µgml^{-1}), green light (225.17 µgml^{-1}) and blue light (220.18 µgml^{-1}). Nostoc sp. BTA80 released minimum extracellular ammonium in white light (103.29 µgml^{-1}) and all other light conditions (red, green and blue). The present findings suggest that Nostoc muscorum BTA950 could be used for commercial production of ammonium when grown under white light. These strains were found to be useful for their utilization in agriculture and industry.

Keywords: Ammonium excretion, Biofertilizer, Cyanobacteria, Light quality, Loktak Lake

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INTRODUCTION

Light is one of the most important environmental factors for photosynthetic organisms. It is used to drive photosynthesis and to regulate growth and development. Changes in the spectral composition of light can affect the rate of quanta transfer to one reaction centre over the other, which in turn leads to a decreased photosynthetic efficiency and damages the photosynthetic apparatus [1, 2]. Light quality has a strong influence in light harvesting system of the cyanobacteria [3, 4]. The chromatic acclimation process allows the cells of cyanobacteria to alter its light absorption characteristics in order to regulate photosynthesis according to light availability in different environments. The physiological and molecular basis of short-term and long-term adaptations under changing light quality have been well characterized [5, 6]. However, the response of cellular functions other than photosynthesis to changing light quality is largely unexplored.

The photosynthetic system of many cyanobacteria is highly adaptable to quality and quantity of light in different environments. The heterocystous cyanobacteria can utilize light energy to support both carbon-dioxide fixation and nitrogen fixation under aerobic conditions [7]. This allows them to produce fertilizer nitrogen that can contribute to the growth of plants. The application of nitrogen fixing cyanobacteria replaces 20-30% of the chemical nitrogen fertilizer demand, increasing the natural fertility of the paddy-field soils [8]. The nitrogen fixed by cyanobacteria is made available to the environment mainly by autolysis and mineralization after death [9]. The use of the conventional chemical farming methods, which substantially increased crop production, was once regarded as a kind of agriculture revolutions which would solve all problems relating to producing sufficient food for the ever growing world population. Increasing prices of agro-chemicals especially nitrogen often leaves farmers with low profit.

The role of cyanobacteria as biofertilizer has been limited to its relevance and utilization in rice crops [10]. In attempting to develop productive, profitable and sustainable agriculture systems, several agriculturalists have been turned to farming methods, which are based on biotechnologies. One of the several approaches to achieve this goal is using the nitrogen fixing cyanobacteria to improve soil fertility and productivity. The use of nitrogen fixing cyanobacteria ensures saving entirely or partially the mineral nitrogen required in crop production. However, our knowledge about chromatic adaptation and the effects of light upon its extracellular ammonium excretion are limited.

In this paper, we report the findings obtained after the responses of cyanobacterial strains under controlled laboratory conditions in attempt to elucidate how changes in light quality affect its growth and extracellular ammonium excretion which could be potential source for biofertilizer formulation.

MATERIALS AND METHODS

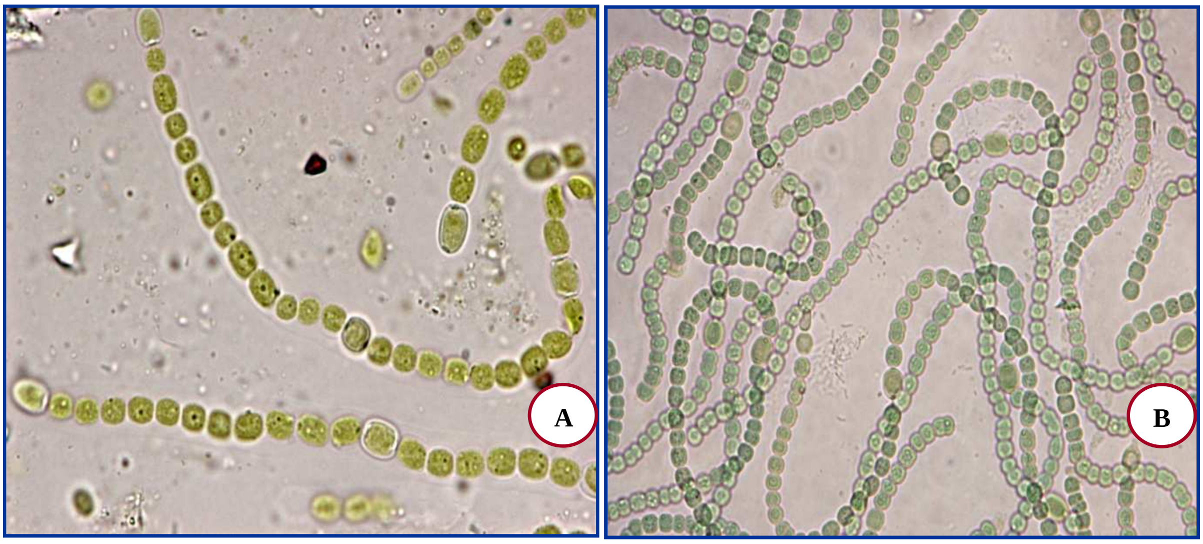
Cyanobacterial strains
Six (06) cyanobacterial strains namely, *Nostoc* sp. BTA60, *Nostoc* sp. BTA61, *Nostoc commune* BTA67, *Calothrix* sp. BTA73, *Nostoc* sp. BTA80 and *Nostoc muscorum* BTA950 used in the present study were obtained from National Repository for Cyanobacteria and Microgreen algae (Freshwater) at DBT-IBSD, Imphal, Manipur, India which were earlier isolated from Loktak Lake, the only largest freshwater lake in the North-Eastern region of India.

Growth conditions
The cyanobacterial strains were grown in 150 ml cotton-plugged Erlenmeyer flasks containing 100 ml of sterile nitrate-free (-N) BG-11 medium [11] and kept in the culture room. Cultures were allowed to grow in light intensity of 54-67 µmol photons $m^{-2}s^{-1}$ provided by cool white fluorescent tubes following 14 h light:10 h dark cycle maintained at 28±2ºC. Chromatic illumination was provided by red, blue and green coloured cellophane papers interposed between the flasks and the fluorescent tubes. One of the flasks was kept in white light uncovered (without cellophane paper) under same experimental conditions and was considered as control. The flasks were shaken manually for two to four times daily to prevent cell clumping, circulation of air and nutrients.

Extracellular ammonium determination
Culture filtrates were collected by filtration of homogenized algal suspension to estimate the ammonium released into the medium. Ammonium concentration in the medium was measured by phenol-hypochlorite method [12]. All the experiments were carried out in triplicates and the data were presented as mean values ± SD of three replicates.

RESULTS AND DISCUSSION

Photomicrographs of cyanobacterial strains used in the present study were presented (Fig. 1).



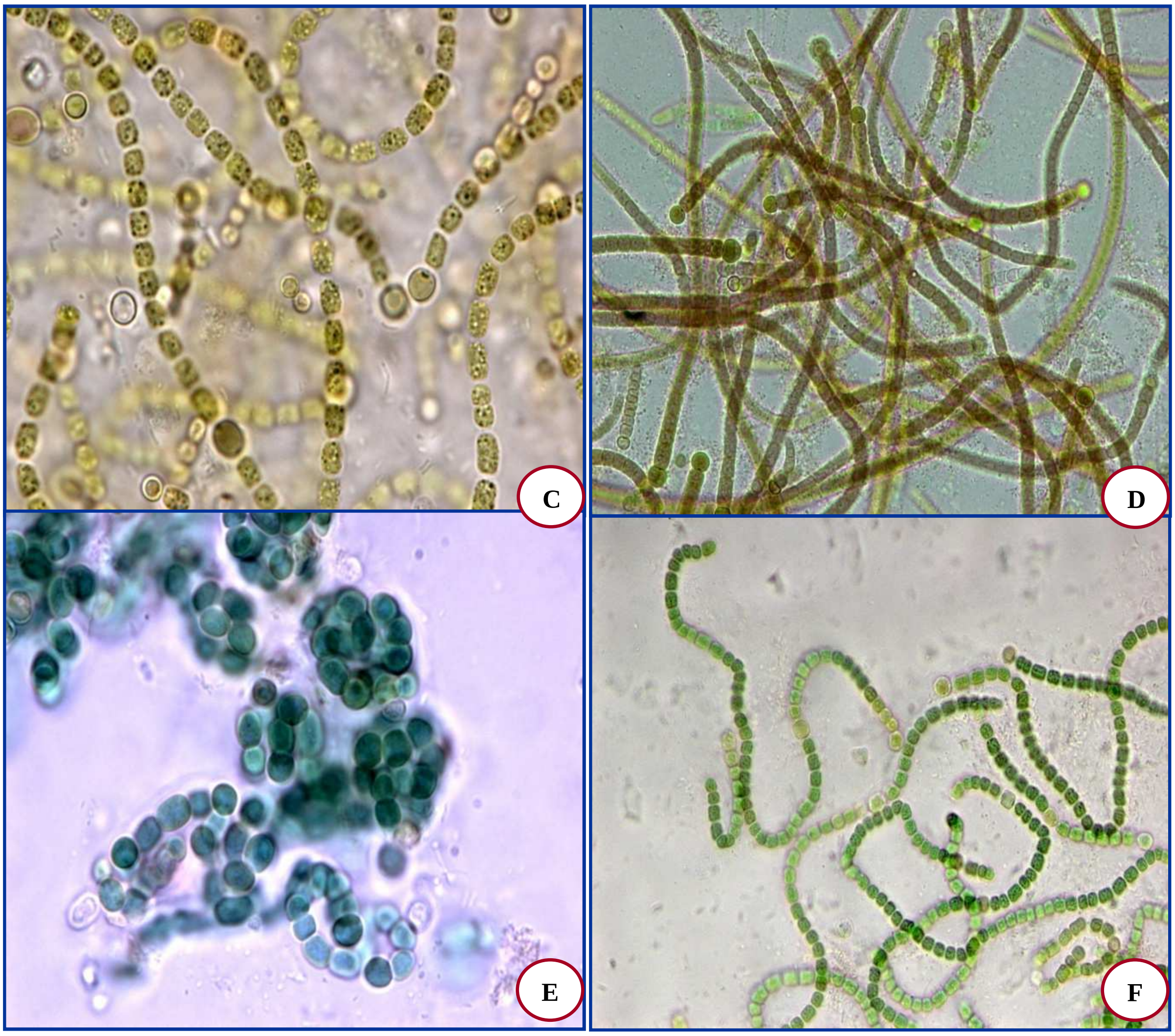
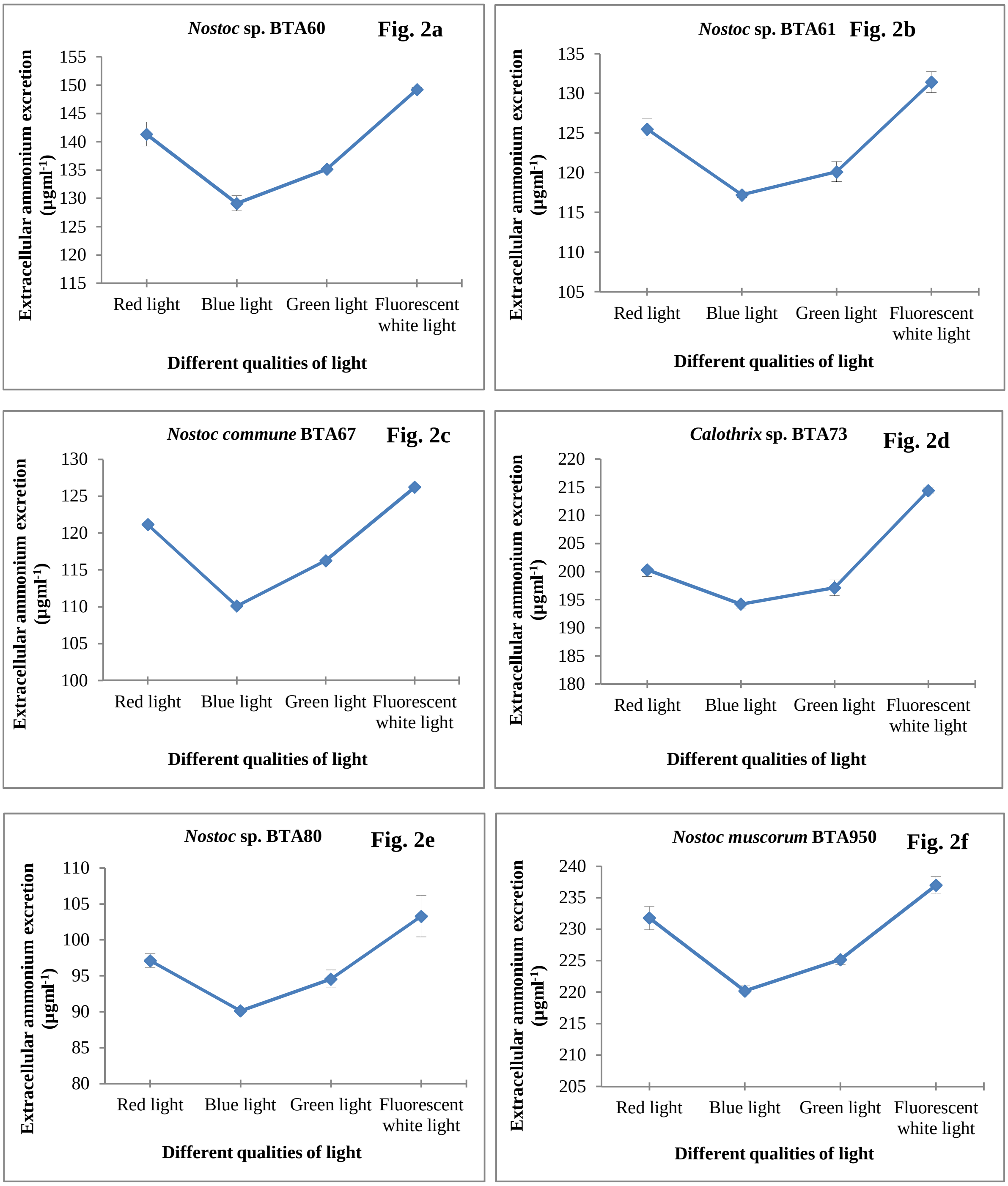


Fig. 1. Photomicrographs of cyanobacterial strains used in the present study
A. *Nostoc sp. BTA60;* ***B.*** *Nostoc sp. BTA61;* ***C.*** *Nostoc commune BTA67;* ***D.*** *Calothrix sp. BTA73;* ***E.*** *Nostoc sp. BTA80;* ***F.*** *Nostoc muscorum BTA950*

In the present study, among the four light qualities used, white light was found to be the most effective on extracellular ammonium excretion followed by red, green and blue light (Fig. 2a-2f). The observed order of preference of light quality by the cyanobacterial strains were white > red > green > blue. *Nostoc muscorum* BTA950 released maximum extracellular ammonium (236.98 μgml^{-1}) in white light followed by red light (231.77 μgml^{-1}), green light (225.17 μgml^{-1}) and blue light (220.18 μgml^{-1}). *Nostoc* sp. BTA80 released minimum extracellular ammonium in white light (103.29 μgml^{-1}) and all other light conditions (red, green and blue).



Figs. 2a-2f. Excretion of extracellular ammonium in different qualities of light

The present findings showed that the ammonium excretion is maximal under white light and is significantly reduced under red, green and blue light. This discrepancy could be due to various reasons e.g. due to differences in species, physiological age and light intensity and nutrient levels. Concentrations of extracellular ammonium excretion for cultures grown under white light were significantly higher than those in cultures grown under all other light colour treatments. There were no significant differences between the red, green and blue light treatments. The ammonium concentration under blue light was significantly lower than those grown under all other light quality conditions. Recently, information about how light quality regulates vegetative cell shape and integrity of cyanobacterial filaments has begun to emerge [13]. When incubated under red, green and blue light, cultures changed pigmentation towards blue, green and red colour. In this study, in green and blue light the culture appeared to remain the same. [14] reported that these strains used in the present study also showed good nitrogenase activity. This findings on ammonium production by cyanobacteria was similar with the earlier report [15, 16]. They have reported extensively on photosynthetic production of ammonia by cyanobacteria.

With the growing realization that chemical based agriculture is unsustainable and is slowly leading to ecological imbalance, the latter part of the last century witnessed the emergence of the concept of ‘‘organic agriculture’’ advocating minimum use of chemical fertilizer and increasing dependence on biological inputs like compost, farm yard manure, green manure and biofertilizers. The choice of biological fertilizer is due to eco-friendly, fuel independent, cost effective and easily availability. Cyanobacteria also add organic matter, synthesize and liberate amino acids, vitamins and auxins, reduce oxidizable matter content of the soil, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates and increase the efficiency of fertilizer use in crop plants [17]. Thus the concept of using N_2-fixing cyanobacteria as nitrogenous biofertilizer is not fully explored and major short comings are still limiting the wide and effective utilization of this bio-fertilization technology.

The findings revealed that ammonium excretion and colour of strain drastically changed in response to light quality through complementary chromatic adaptation. Cyanobacteria species are recommended to be used as biofertilizers instead of utilizing the expensive industrial chemical fertilizers. This is because of the increased cost of chemical fertilizers that cause soil and water pollution. In comparison, cyanobacteria are a cheap source of N, which does not cause pollution. Data generated during the present investigation could be useful in understanding of a commercial or biotechnological potential of these strains. Ammonium excreting cyanobacteria are useful for many purposes.

CONCLUSION

In summary, white, red, green and blue colour of light was employed for the production of extracellular ammonium from freshwater cyanobacterial strains from Loktak Lake. A maximal ammonium excretion was observed when grown under white light. Only a few cyanobacterial species were found to be chromatically adaptable and a very few have been known to produce high ammonium under variable light quality employing chromatic adaptation. Improvement in the ammonium excretion with changes of environmental factors (especially different qualities and intensity of light) could be a good basis for the exploitation of studied cyanobacterial strains as a source of valuable products. The present findings indicate that producing mass culture of potent cyanobacterial strains would attract attention for biotechnological purposes. These potent strains could be useful for their utilization in agriculture and industry. This study would offer an excellent scope for the exploitation of such strains as good biofertilizer, generating valuable nitrogen fertilizer.

Acknowledgements
The authors thank to the Director, DBT-IBSD, Imphal, Manipur, India for providing laboratory facilities and required infrastructure. The authors acknowledge the financial grant provided by Indian Council of Agricultural Research (ICAR), Government of India.

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