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ISOLATION AND CHARACTERIZATION OF AMMONIA EXCRETING CYANOBACTERIUM *CYLINDROSPERMUM* SP. NDOP002

Om Prakash and N. Dwivedi*
Department of Botany, U.P. College (Autonomous), Varanasi, India
*Email: drnagendra.dwivedi@gmail.com

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ABSTRACT

Cylindrospermum sp. NDOP002 was isolated from agricultural fields of Azamgarh, U.P., India. It was characterized by morphological methods. Total chlorophyll a content was 18.5 µg/ mg dry weight. Organism achieved a stationary phase of growth after 15 days of cultivation. Ammonia excretion was monitored from 6 hrs. of culture to 20 days. The ammonia content of culture increased up to 7 days (Maximum amount of 3.23 µg/ ml) and then decreased. Approximately a constant amount of ammonia was maintained from 9th and onwards days of growth. Maximum glutamine synthetase (GS) activity of 8.33 mM γ-glutamyl hydroxamate min-1 mg chl-1 was observed on the 5th day of culture and then decreased. Approximately constant GS activity was observed after 9th and onward days of growth. Algalisation experiment showed an increase in growth parameter of rice plant in algalised set. Length of roots was 6.4-6.9 cm in non algalised and 7.2-7.6 cm in algalised rice plants whereas the length of shoots was 5.7-6.1 cm in non- algalised and 9.5-10.1 cm in algalised rice plants. The amount of ammonia in algalised set was 2.1 µg/ ml on the 10th day of the experiment. Experimental findings clearly suggest *Cylindrospermum* sp. NDOP002 as suitable inocula for algalisation of rice fields of Azamgarh district, U.P., India.

Keywords: Cyanobacteria, *Cylindrospermum* sp. NDOP002 and Ammonia

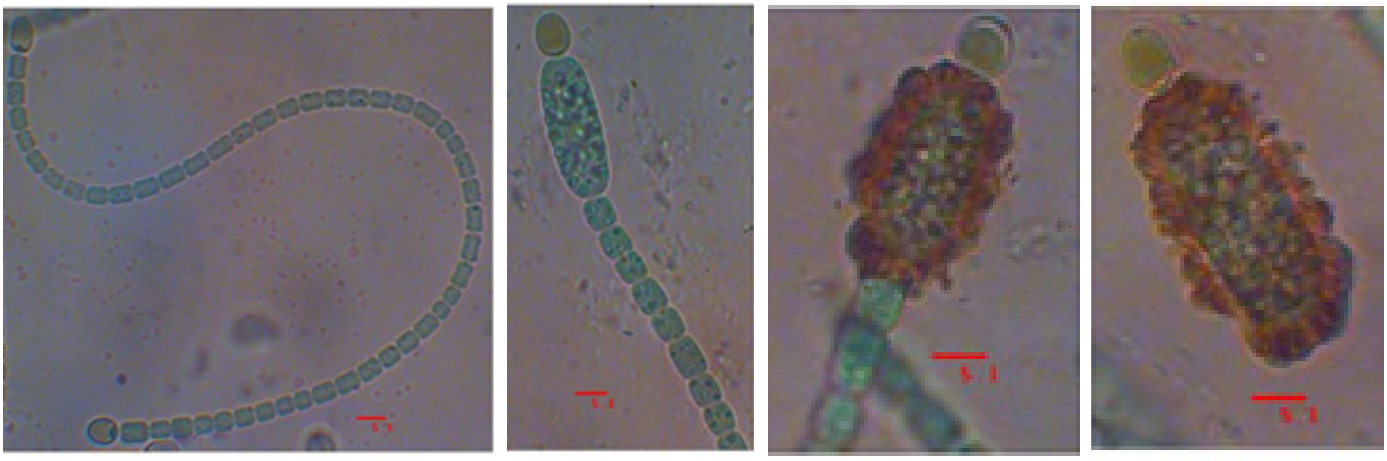
INTRODUCTION

The contribution of cyanobacteria in enriching paddy fields particularly nitrogen content is well established. The Paddy field ecosystem is known to support the luxuriant growth of cyanobacteria. Besides, providing fixed nitrogen cyanobacteria also improves plant growth by secreting growth regulators, siderophores, etc. The abundance of cyanobacteria was recorded more in tropical and subtropical regions compared to other regions of the world. Wide variation in cyanobacterial abundance was noticed in rice fields i.e. 75% of the total algal flora of Indian rice fields (Pandey, 1965), 86% of the total algal flora of southern Iraq (Al-Kaisi, 1976), 70% of algal flora if Italian soil (Materasi and Balloni, 1965). Algalisation of fields with cyanobacteria has shown an increase in nitrogen content up to 14% (Rao and Burns 1990, Singh and Bisoyi, 1989). Nitrogen fixation of 15-53 kg/ hectare (h)/ year(y) have been observed by cyanobacteria (Kaushik, 2014). Filamentous heterocystous forms are well-known biological nitrogen fixers. Several non-heterocystous forms i.e. *Gleoecapsa* (Wyatt and Silvery, 1969), *Plectonema boryanum* (Stewart and Lex, 1970), *Trichodesmium* sp. (Carpenter and Price, 1976) have also been reported to fix nitrogen fixation. Some species of heterocystous cyanobacteria i.e. *Nostoc*, *Anabaena*, *Cylindrospermum*, *Anabaenopsis*, *Scytonema*, *Calothrix*, *Stigonema*, *Tolipothrix*, *Aulosira*, *Mastigocladus*, *Fischerella*, *Gleoetrichia*, *Hapalosiphon*, *Chlorogloeopsis*, *Campylonema*, *Rivularia*, *Nostochopsis*, *Chlorogloea*, *Schytonematopsis*, *Westiellopsis*, and *Wollea* are efficient nitrogen fixers (Venkataraman, 1993). Some cyanobacterial strains i.e., *Aulosira fertilissima*, *Anabaena variabilis*, *Nostoc muscorum*, and *Tolypothrix Tenuis* are being used in algal biofertilizer technology (Kaushik, 2014). Most of the fixed nitrogen of cyanobacteria is released only after decomposition and autolysis (Martinenz, 1984). The majority of cyanobacterial strains release an insignificant amount of ammonia during their growth period (Martinenz, 1984). Search for continuous ammonia secreting cyanobacterial strains are one of the primary goals of plant biologist. The cyanobacterial strain was isolated, characterized, and studied for ammonia secreting properties.

MATERIALS AND METHODS

Cyanobacterium was isolated from soil collected from agricultural fields of Azamgarh, U.P., India following procedures as mentioned by Mishra *et al.,* 2019. The cyanobacterial strain was purified by repeated streaking method and grown in BG-11 medium without nitrogen supplementation. It was maintained in a culture room set at 28±20c and illuminated by the fluorescent tube of 40w with 14: 10 light and dark cycle.

Morphological parameters as followed by Mishra *et al.,* 2019 were used for the identification of cyanobacterium. The morphological parameters of cyanobacterium were studied by viewing at 400x and 1000x of Olympus 21Xi microscope. Morphological characters were analyzed by Magnus PRO Micro measurement & Image analysis



A B C D

Figure 1. Photomicrograph of *Cylindrospermum* sp. NDOP002 (Scale bar=5.1 µm). A, Young filament with apical heterocysts. B, Filament with young spore. C, Filament with mature spore, D, Mature spore.

software. The strain was assigned to cyanobacterial species following taxonomic descriptions of Desikachary, 1959 and Rippka *et al.,* 1979.

Growth was measured by monitoring chlorophyll content at the interval of 24 hrs. for 20 days. Chlorophyll a was measured by the method prescribed by Myers and Kratz (1955). The phenol hypochlorite method (Solorzano, 1969) was used to measure extracellular ammonia release. 50 ml of cyanobacterial extract was mixed with 2 ml of sodium nitroprusside solution, 2 ml of phenol solution, and 5 ml of oxidizing reagent, blue color developed after 1 hr of incubation. The optical density of blue color was measured at 640 nm. The standard graph was used to calculate the amount of ammonia in the extract. Shapiro and Stadtman (1970) method was used to measure the activity of Glutamine synthetase. The Glutamine synthetase activity is expressed as mM $^{\gamma}$- glutamyl hydroxamate formed min-1 mg chl-1.

Autoclaved sand was placed in two sets of autoclaved Petri-plates and each was moistened by 25 ml of autoclaved tap water. Ten seeds of rice (Kaveri variety) were placed in each set. Both set were placed in incubator for 36 hrs. at 30^0C. One set was algalised by 1 ml of cyanobacterial culture and then both Petri-plates were placed in a growth chamber illuminated with 14:10 light and dark duration. Growth parameters i.e. length of radical and plumule and extracellular ammonia content was measured after 10 days of growth.

RESULTS AND DISCUSSION

Cyanobacterium is filamentous, heterocystous, and unbranched. Heterocyst is on both ends of filament followed by spore (Fig.-1). These are basic morphological features of genera *Cylindrospermum* (Desikachary, 1959) Hence, this cyanobacterial strain belongs to genera *Cylindrospermum*. The filament is blue-green in color, vegetative cells cylindrical (4.89-7.12 µ length and 4.51-4.78 µ breadth) with deep constriction on cross walls (Fig.-1). Heterocyst spherical, subspherical to cylindrical (Fig.-1) with 5.07-9.05 µ length and 4.64-6.43 µ breadth. Spores cylindrical and elongated (Fig.-1) with 18.42-25.53 µ length and 8.97-10.27 µ breadth. Morphological features closely resemble with *Cylindrospermum indicum*, *Cylindrospermum goraphpurense* and *Cylindrospermum alotosporum* (Desikachary, 1959). The shape and size of the spore are quite different as per known species of *Cylindrospermum* (Desikachary, 1959). The cyanobacterial strain may be new species but further characterization is needed. Hence, this cyanobacterium is being identified as *Cylindrospermum* sp. NDOP002.

Growth was monitored by measuring chlorophyll a content on alternate days for 20 days ((Fig.-2). Stationary growth phase was obtained after 15 days of cultivation period ((Fig.-1). Total chlorophyll a content was 18.5 µg/ mg dry weight in the stationary phase of growth.

Ammonia excretion was monitored from 6 hrs. of culture to 20 days. The minimum amount of 0.74 µg/ ml ammonia was observed after six hrs. of culture and ammonia content increased up to 7 days with the maximum amount of 3.23 µg/ ml and then decreased and got near about constant with slight fluctuation after 9th days of growth (Fig.-3). Nitrogen plays a very important role in the growth and productivity of plants. The nitrogen demand of crops is fulfilled by inorganic nitrogen fertilizer and biological nitrogen fixation. Bacteria, Actinomycetes, and cyanobacteria are known for biological nitrogen fixation. Rice field ecosystem favors luxuriant growth of cyanobacteria. Free-living and symbiotic cyanobacteria (*Azolla-Anabaena* complex) are known to enrich the nitrogen content of rice fields. Enzyme complex nitrogenase reduces dinitrogen to ammonia. Nitrogenase is oxygen-sensitive and located exclusively in the heterocyst of cyanobacteria (Wolk and Wojciuch, 1971). Most fixed nitrogen of free-living cyanobacteria is made available to plants only after autolysis and decomposition of cyanobacteria. Biological fixation occurs in heterocyst with help of nitrogenase. Ammonia formed in heterocyst

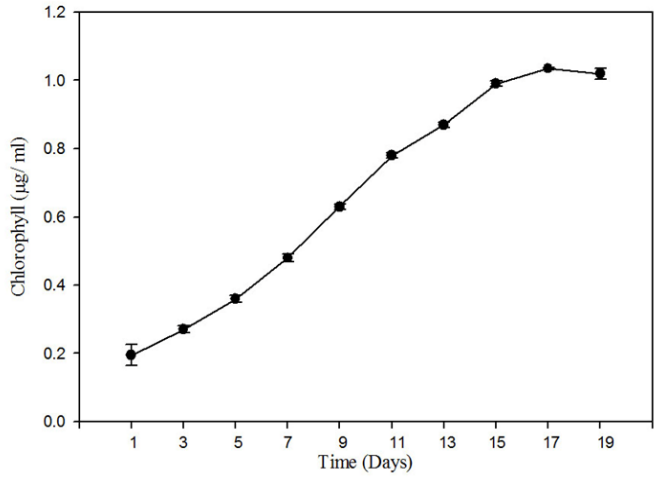


Figure 2. Growth behavior of *Cylindrospermum* sp. NDOP002. Values are mean of triplicate ± S.D., bars indicate standard deviation.

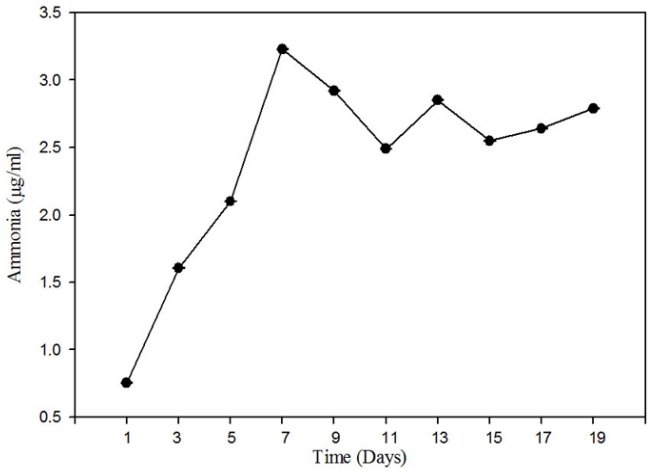


Figure 3. Ammonia excretion by *Cylindrospermum* sp. NDOP002. Values are mean of triplicate ± S.D., bars indicate standard deviation.

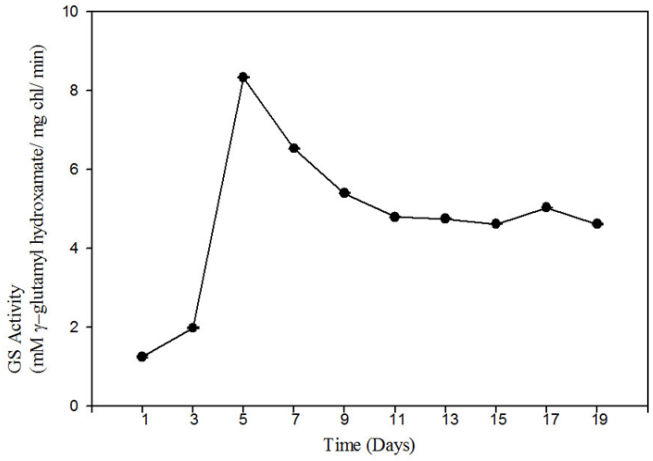


Figure 4. Glutamine synthetase activity of *Cylindrospermum* sp. NDOP002. Values are mean of triplicate ± S.D., bars indicate standard deviation.

Figure 5. Effect of algalisation on rice seedling growth. A, Non- algalsed seedling. B, Algalised seedling.

is assimilated to Glutamine by enzyme Glutamine synthetase and then in this form of nitrogen moves to surrounding vegetative cells. Unassimilated Ammonia diffuses naturally from heterocyst so GS activity plays determining role in ammonia excretion in diazotrophs. Cyanobacteria with low GS activity are known to excrete ammonia. *Cylindrospermum indicum* NDOP001 is continuously excreting ammonia in culture medium with a maximum amount of 3.23 µg/ ml of ammonia on the 7th day of culture (Fig.-3). This may due low rate of ammonia assimilation in comparison to ammonia formation.

GS activity was also monitored for 20 days. GS activity increased from 1st day and maximum activity of 8.33 mM ᵞ- glutamyl hydroxamate formed min-1 mg chl-1was noticed. GS activity decreased from the 5th day onward and got near about constant after the 9th day (Fig.-4). Even with this pattern of GS activity the continuous presence of ammonia was noticed in the culture medium. This showed lesser assimilation of ammonia in comparison to its formation.

Algalisation experiment showed an increase in growth parameter of rice plant in the algalised set (Fig.-5). Length of roots was 6.4-6.9 cm in non-algalised and 7.2-7.6 cm in algalised rice plants whereas the length of shoots was 5.7-6.1 cm in non-algalised and 9.5-10.1 cm in algalised rice plants (Fig.-5). The amount of ammonia in the treated set was 2.1 µg/ ml on the 10th day of experiment. Growth of rice seedling was more in algalised set comparison to the non-algalised set. This clearly indicates growth promotion by ammonia excreting strain of cyanobacteria. One of the primary objectives of algal biotechnology is to search continuous ammonia excreting cyanobacterial strains so this cyanobacterium may prove suitable inocula

for algalisation of rice fields of Azamgarh district of U.P., India.

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