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Ammonium translocation in Cyanobacteria and their possible use as Biofertilizers.

Interim Report No-2

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A visit to the philippines of Dr S. Boussiba toward the midle of January is planned. The purpose of this visit is to exchange information regarding work in progress and to plan for future collaboration- visits of students to the laboratory at Sede Boker. A detailed working plan for Dr Boussiba's visit to the Institute of Biological Science at Los Banos is enclosed .(Appendix no-1)

Task No-1. Selection of organisms and optimization of growth conditions.

Growth conditions.

Laboratory cultures. Algae were cultivated in BG11o medium either in 100 ml in 250 ml flasks placed on a gyratory shaker under constant illumination of 75 $\mu E.m^{-2}. s^{-1}$ at a constant temperature of 27°C; or in 500ml glass tubes, into which either air or 1.5% CO2 in air were bubled , and which were placed in a water bath at a constant temperature of 28°C. The light intensity at the surface of the vessels was 175 $\mu E. m^{-2} s^{-1}$. Illumination was provided by 20-W coolwhite fluorecent lamps.

Outdoor cultures. 2.5 m^2 oval-shaped ponds with two channels forming a single loop were used. The culture, 250 liter in volume (medium was BG-110) and 10 cm in depth, was stirred by a paddle wheel. CO_2 was supplied to maintain the pH at a range of 6.5 - 7.5.

Enzymes assays . Nitrogenase activity was estimated by the acetylene reduction method (Stewart et al., 1967). 4.6ml samples of algal culture prewashed in fresh BG-11o medium were placed in a 25ml Weaton bottle sealed with a flanged rubber septum. The weaton bottles were subjected to rotary shaking and illuminated with a quantum flux of 75 $\mu E\ m^{-2} s^{-1}$, during the assay. Cell suspensions were allowed 10 min of acclimation before injection of C_2H_2 . To begin the assay , 2.5 ml of air were withdrawn and replaced with 2.5 ml acetylene. Etylene was analyzed on a H.P. 5880A gas chromatograph using a stainless steal column packed with Poropack-N(0.2 cm i.d. , 265cm length).

Results

1. Work carried out at Sede Boker with the Blue-Green Algae (BGA) isolated from the Phlippines, Nostoc sp- Cy 24.

Effect of pH on the growth and nitrogenase activity of *Nostoc sp.*

Nostoc sp was grown in different pH's as described in Fig1. Optimal growth was observed at pH 10 and the calculated doubling time at this pH was 18 h. Nitrogenase activity was not affected by the changes in pH in the growth medium (Table1).

2. Outdoor mass production of Anabaena azollae

The fast growth rate of this organism observed in the laboratory, the possibility of obtaining dense cultures up to 30-40 mg chl/liter (equivalent to 1.5-1.75 gr dry weight liter-1), its broad temperature tolerance , and its ability to grow almost at optimal values to fix nitrogen over a wide range of pH with a relatively small reduction in activity prompted us to test its potential for mass production outdoors as a nitrogen biofertilizer. Some characteristic features of outdoor cultivation of this strain were observed: 1) Relatively small amounts of ammonia were present in the medium during growth (between 0.1 and 0.3 mM). Whether this feature represents un induced release of ammonia, as also observed by others (1), or simply indicates decomposition of living cells, is not yet known. This phenomenon was not observed in the laboratory. 2) Significant reduction in frequency of heterocysts (5-7%) and nitrogenase activity (4-7 µmol C_2H_4 mg l^{-1} chl h^{-1}) during growth, compared with laboratory cultures. 3) Sensitivity to solar irradiance which necessitate continuous shading of the pond in the summer (August), reducing light intensity by 30%.

Data accumulated during several seasons of the year at Sede-Boker are presented in Table 2.

A major effect which controlled the mass production of this nitrogen fixing strain was the temperature fluctuation during the several months of production.

Maximum yield of 17.9 $m^{-2} d^{-1}$ was obtained during the month of August in which the morning and noon temperatures were close to optimum . This relatively high rate of production obtained with Anabaena azollae, compared with the rate of production of 6-8 g $m^{-2} d^{-1}$ obtained in other systems and different strains (2) , points to the potential of this isolate for production and use as a nitrogen biofertilizer. About 10 kg (AFDW) of algae are need (3) to inoculate 1 ha of rice field, thus up-scaling of this system to a larger 10 m^2 pond will require only about two months of production in the hot season to achieve sufficient algal material to inoculate 1 ha. This kind of operation could therefore be advantageous for small areas of rice production in which cyanobacteria are applied as substitute nitrogen fertilizers.

3. The release of ammonia to the surrounding environment

The accumulated nitrogen in the algal cells can be made available to the rice plants mainly by mineralization when the algal cells are microbially decomposed, only a small part of this nitrogen being available through exudation when the algae are still alive(1). During the course of growth in outdoor ponds a sudden drastic drop in temperature occurred (below 10 C). This caused rapid decomposition of the Anabaena cells and the content of ammonia in growing medium rose (Fig. 2).

The released ammonia was consumed and promoted the establishment of new species of algae revealed by microscopic observation and the total loss of nitrogenase activity(Fig.2). This situation is comparable to the decomposition of cyanobacteria in rice fields, when the nitrogen compounds are utilized by the rice plants.

References

1. Martinez, M.R., Algae: biofertilizer for rice. Philippines Council for Agriculture Research and Resources Development (PCARRD), PCARRD Monitor, 1984, 12, 9.
2. Watanabe, A., On the mass culturing of a nitrogen fixing blue-green algae Tolypothrix tenuis. J. Gen. Appl. Microbiol., 1959, 5, 85-91.
3. Roger, P.A., Watanabe, I., 3. Techniques for utilizing biological nitrogen fixation in wetland rice: potentialities, current usage, and limiting factors. Fertilizer Research, 1986, 9, 39-77.

Table1. Effect of pH on nitrogenase activity of Nostoc sp.

pH	Nitogenase activity *
6.0	4.06
7.0	3.95
8.0	4.44
9.0	3.60
10.0	3.85

* nitrogenase activity-µmol ethylene/mg chl h

Table 2. Outdoor production of Anabaena azollae in 2.5 m2 ponds

Month (1987)	Temperature (oC)		Output rate ($g\,m^{-2}\,d^{-1}$)	Nitrogenase* activity	Heterocysts (% of total cells)
March	12-15[1]	23-26[2]	5.6	4.7	4-5
June	18-21	28-31	12.3	5.2	5-6
August	21-24	31-34	17.9	5.8	5-6

1- morning; 2-noon
*- nitrogenase activity-µmol ethylene/mg chl h

Figures Legends:

Figure 1. Effect of pH on the growth of Nostoc sp.

Nostoc sp cultivated in BG11o and adjusted at different pH's with the following buffers : pH 6 and pH 7.0 adjusted with 20mM HEPES; pH 8 adjusted with 20mM posphate ; pH 9 and pH 10.0 adjusted with 20mM carbonate/bicarbonate.

Figure 2. Growth and ammonia release of Anabaena azolla cultivated in 2.5 m^2 pond.

see materials and methoa for mode of cultivation.

0----0 ammonia

□---□ chlorophyll

arrow indicate the drope of temperature bellow 10°C

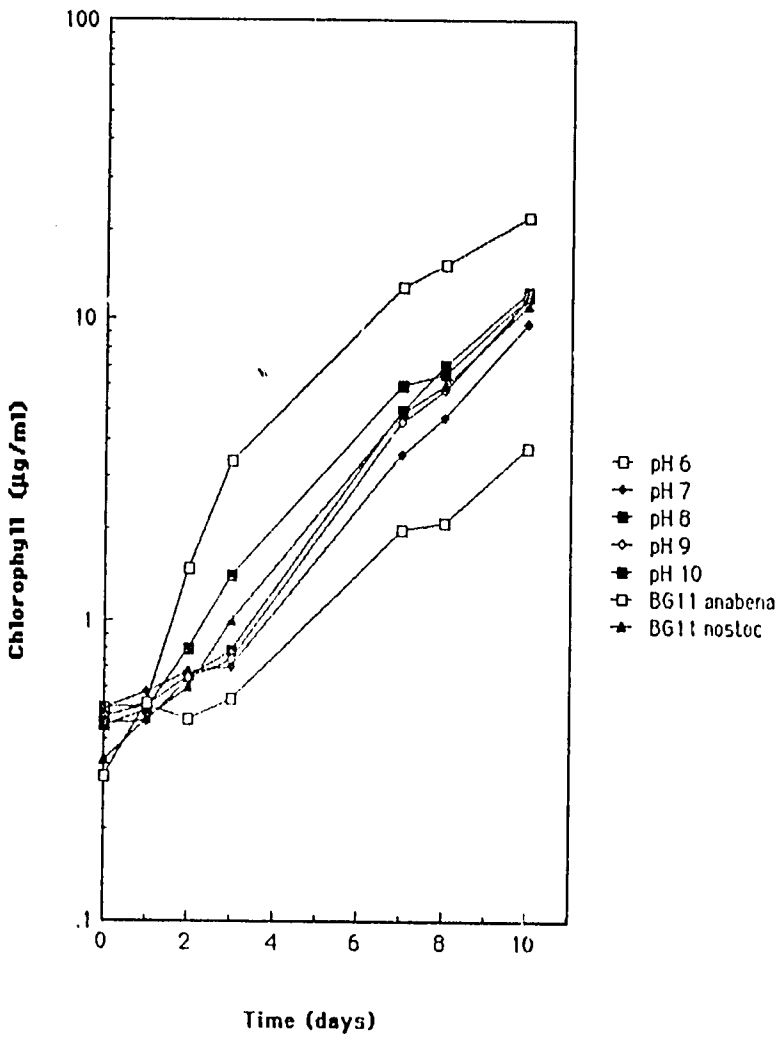

Fig 1. Effect of pH on the growth of Nostoc sp

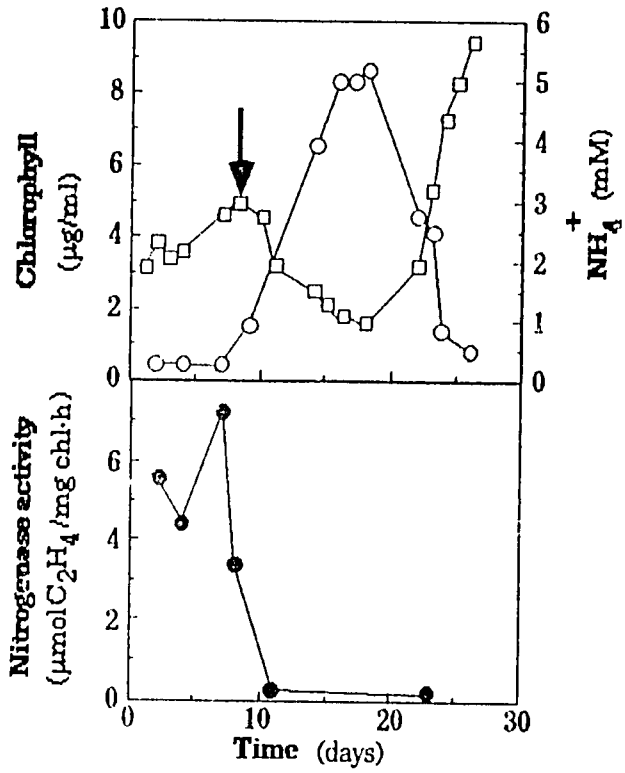

Figure 2. Growth and ammonia release of Anabaena azollae cultivated outdoor in 2.5 m^2 pond.

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January 15, 1988	- Friday	- Arrival Lodging at UPCO Guest House, Tel. #22-23
16	Sat.	- Rest Lunch - Dinner at Guest House with Research Personnel of the Project
17	Sunday	- Trip around the campus, ponds Dinner with Milagrosa R. Martinez
18	Monday	Courtesy calls at DFI, IBS, CAS, BIOTECH Seminar BIOTECH Dinner with DFI & IBS staff
19	Tues.	- Trip to IBS & BIOTECH and setting up of Turbidostats Dinner with BIOTECH
20	Wed.	- Consultation on laboratory culture studies on BGA
21	Th.	- Seminar IRRI
22 23 24	Fri. Sat. Sun.	- Trip to Pangasinan and Baguio [illegible]
25	Monday	- Consultation on the turbidostat
26	Tues.	- Consultation on the pond set-up
27	Wed.	- Designing and requisitioning of materials for the ponds
28	Thursday	
29	Friday	- Seminar IBS
30	Sat.	- Trip to the South
31	Sunday	- Departure

DFI - Development Foundation, Inc. (the one in-charge of disbursement of AID funds)
IBS - Institute of Biological Sciences
CAS - College of Arts and Sciences
IRRI - International Rice Research Institute
BIOTECH - National Institutes of Biotechnology and Applied Microbiology