PD-AAW-286
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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 7/24/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. CS-233

Attachment

2 cys

PD-AAW-286

CDR Project No C5-233

AID-CDR Grant No. DPE -5544-G-SS-6036-00

Ammonium translocation in Cyanobacteria and their possible use as Biofertilizers.

Interim Report No-1

Principal investigators:

Dr. S. Boussiba., Prof A. Richmond

Jacob Blaustein Institute For Desert Research

Ben Gurion University of the Negev at Sede Boker Campus

84993 Israel

Collaborating investigators

Dr. M. R. Martinez., Prof P.C. Payawal

Institute of Biological Science

University of the Philippines at Los Banos

College, Laguna 3720 Philippines

Task No. 1

Selection of organisms and optimization of growth conditions.

1. The visit of Dr Martinez at the laboratory of Algal Biotechnology at Sede Bok December 15 to January 3rd.

We have obtained from Dr. Martinez, 6 nitrogen fixing Blue-Green algae isolat from rice fields in the Philippines.

The species are coded as follows:

1) *Nostoc* - *Cy 41*

2) *Gleotrichia* - *Cy 22*

3) *Hapalosiphon* - *Cy 23*

4) *Tolypotthrix* - *Cy 34*

5) *Anabaena* - *Cy 1*

6) **Nostoc sp*- *Cy 24*

* We thank Dr P.A. Roger from the international Rice research institute at Lo Banos for the permission to work with this isolate.

During Dr Martinez visit's we have outlined a protocol which will serve as a fram work to test, the potential use of the different N_2 fixing Blue Green Algae (BGA, se appendix no-1) as nitrogen biofertilizer. Effort has been made also to cultivate som of these BGA in BG_{110} media.

2. Work carried out at SEDE BOKER with the Philippines BGA isolates.

The six isolates of BGA have been inoculated into agar plates (1.5% in BG_{110} medium and were thereafter repeatedly transferred every two weeks. It was essential to obtai these algae liquid cultures free as much as possible from contaminants, prior t preforming physiological studies. When this stage was completed we started t cultivate two isolates: Cy 1 and Cy 24 were grown in liquid cultures in BG11 medium in a guiratory shaker under controlled growth conditions(30 C, continuous illumination at light intensity of 75 $\mu E\ m^{-2}\ s^{-1}$). The performance of these isolates is being tested according to the protocol constructed

3. The performance of *Anabaena azollae-AET-325*

This strain which was isolated from Azolla filiculoides was donated by Prof. Elisha Tel-Or, Faculty of agriculture The Hebrew University at Rehovot , Israel.

Preliminary observations revealed; relatively high frequency of heterocysts up to 12%; high growth rate as indicated by a doubling time oh ca. 11h and excretion of ammonia in the presence of MSX with Glutamine synthetase being partially still active. This is in contrast with results obtained for other BGA; N fixing and non fixing and we were thus much encouraged to further inquire about the potential use of this strain as nitrogen biofertilizer.

Methods

Growth conditions. The algae were cultivated in 500 ml sterilized glass columns inside a transparent plexiglass circulating water bath. The thermostat- controlled water temperature was 30^0 C. A constant photon flux of 175 $\mu E\ m^{-2}\ s^{-1}$ at the surface of the growth vessel was supplied laterally from a battery of 8 cool-white florescent lamps. Continuous aeration was provided by bubbling filtered air containing 1.5% CO_2. The standard growth medium was BG-11o (Stanier et al., 1971). Unless otherwise stated culture samples were remove during the logarithmic growth phase and used for the different experiments described bellow.

Ammonium exudation trials.

Ammonium exudation was induced by the addition of methionine sulfoximine (MSX) a specific and irreversible inhibitor of the enzyme glutamine sythetase (G.S). Cells withdrawn from logarithmic growing cultures were centrifuged and the pellet washed once with BG-11o and supplemented with 20mM phosphate buffer. Suspensions containing 0.3 to 0.5 mg cell protein $.ml^{-1}$ were incubated at 30^0C. At intervals samples were centrifuged and the amount of ammonia released was determined by Soloranzo's method (1969).

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-110 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)

Other methods Chlorophyll a was determined by measuring absorbance at 663 nm using an extinction coefficient of 0.82 (Mckinney 1941). Protein was determined according to the procedure of Lowry et al., (1955). Heterocysts frequencies were calculated from microscopic observations and are expressed as % of the total number of cells in the cultures.

The effect of temperature on growth was studied in a temperature block maintaining a temperature gradient of 20 to 35 with 1.5 C degrees increments between adjacent test tubes. The light intensity at the bottom surface of the tubes was 110 $\mu E\ m^{-2}\ s^{-1}$.

Experimental data

a) Determination of the optimal temperature for growth-Fig-1

b) Nitrogenase activity and heterocysts frequency during growth-Fig-2

c) Effect of pH on growth and nitrogenase activity -Fig-3

d) Effect of NaCl on nitrogenase activity-Fig-4

e) Effect of pH on ammonium excretion-Fig-5

Conclusions

The data obtained so far with this strain point out on its potential use as a nitrogen biofertilizer. This strain grows relatively fast (μmax of 0.067 h^{-1}), and can grow almost optimally over a wide range of pH's (6.0 to 9.0 pH).

It posses a high rate of nitrogenase activity which is not much affected by exogenous pH's.In the presence of MSX this strain excretes ammonia at a high rate which correlate with its nitrogenase activity. Also, it has some resistance to salinity and can tolerate a broad range of temperatures. Since rice paddies undergo environmental changes such as fluctuation in pH , temperature and salinity during there development , BGA which can tolerate such changes could serve as efficient biofertilizers.The data described here regarding the AET 325 strain can be therefore considered advantageous , for the use of this strain as biofertilizer.

This strain will be cultivated outdoors in 2.5 m^2 ponds as a further step in testing its potential as nitrogen biofertilizer.

Literature cited

1) Stanier, R.Y., R. Kunisawa, M. Mandel and G. Cohen-Bazir, Purification and properties of unicellular blue-green algae (under Chroococcales). Bacteriol. Rev., 171-205 (1971).

2) Solorzano, L., Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14, 799-801 (1969).

3) Stewart, W.D.P., G.P. Fitzgeral and R.H. Burris, In situ studies on N2- fixation using the acetylene reduction technique. Proc. Nat. Acad. Sci. USA 58: 2071-78.

4) McKinney, G., Absorption of light by chlorophyll solutions. J. Biol. Chem., 140, 315-22 (1941).

5) Lowry, O.H., N.J. Rosenbrough, A.L. Farr and B.J. Randall, Protein measurements with the folin phenol reagent. J.Biol .Chem 265-275(1951)

Appendix No. 1

Criteria Affecting Growth, N_2 Fixation and NH_4^+ Excretion of Cyanobacteria.

I. Measurements of growth, N_2 fixation and NH_3 exudation

1)* Determination of maximum growth (μ max)

2)* Growth as a function of N_2 fixation

3)* Growth as a function of NH_3 exudation - induced vs non-induced

4)* GS activity vs NH_3 excretion

II. Environmental Factors

1) Light intensity and quality

2) *Temperature

3) Relative humidity: amount of rainfall - intensity of rainfall

4) *Agitation of mixing

5) *Harvesting methods

III. Chemical/Nutritional Factors

1) *pH

2)* Salinity - NaCl

3) Fe

4) Mo

5) P

6) Carbon source and amount

7) Effect of fertilizers

urea

ammonium sulphate

super P

8) Effect of pesticides

9) Effect of herbicides

*Experimental factors in the framework of this project

Fig 1. Determination of optimal growth temperature.

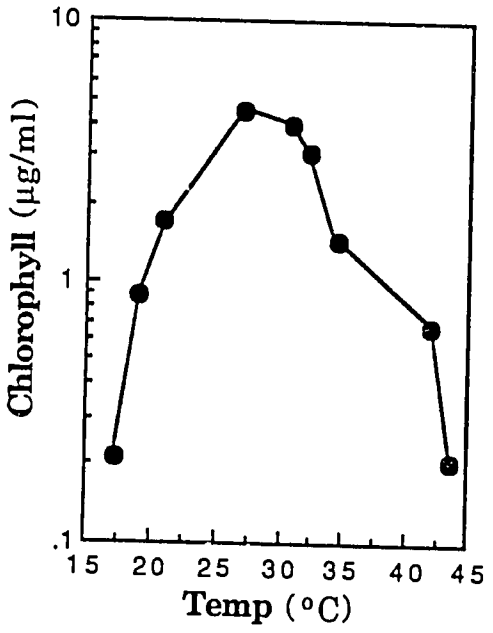

Fig 2. Nitrogenase activity and heterocysts frequency during growth

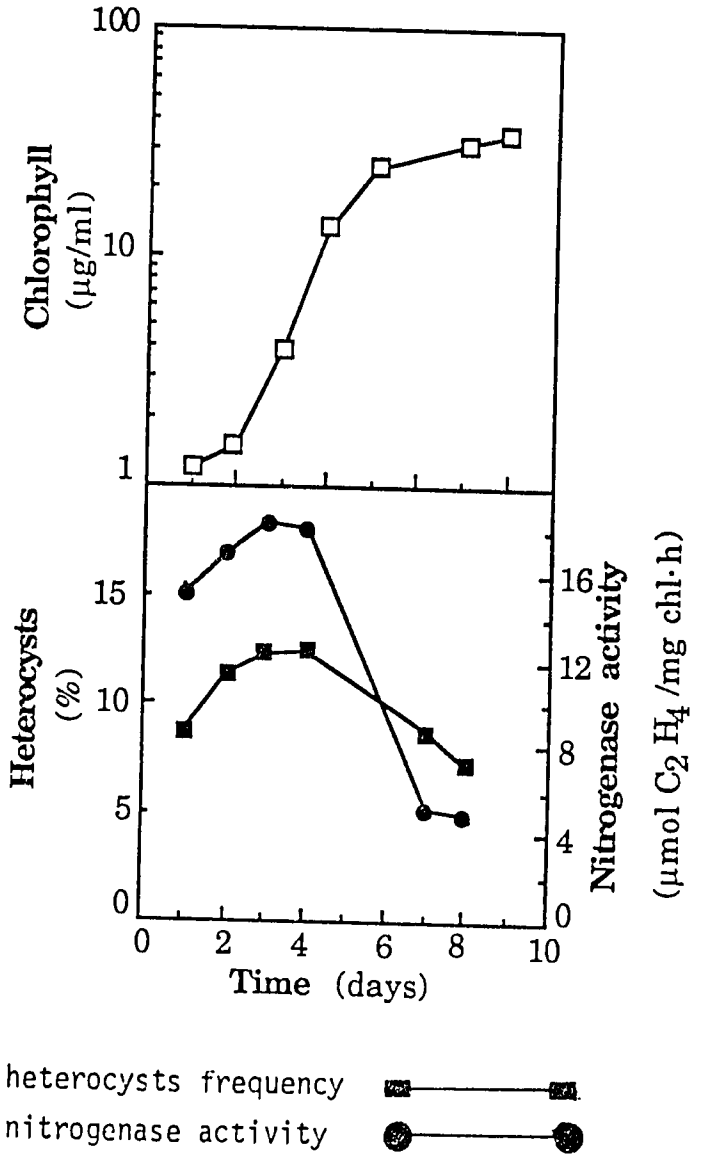

Fig 3. The effect of pH on the growth rate and nitrogenase activity

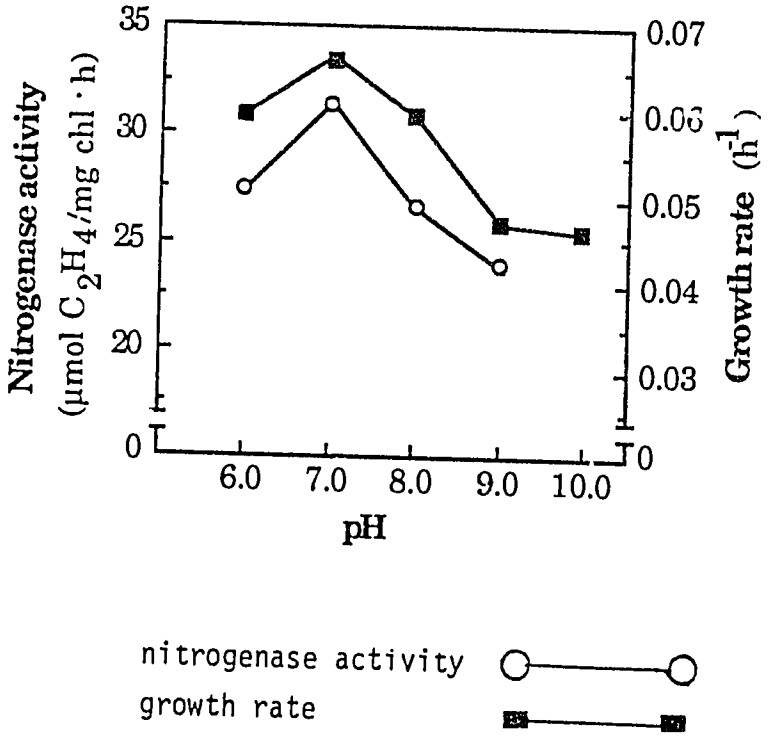

Fig 4. Effect of pH on ammonium excretion and nitrogenase activity

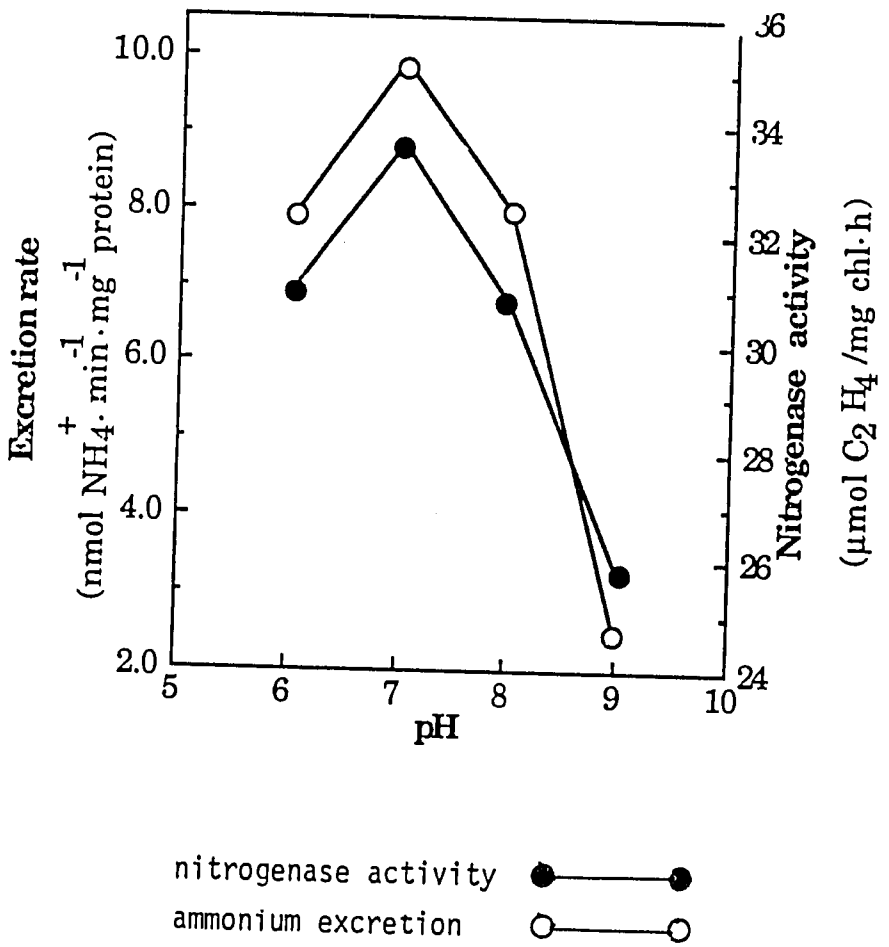
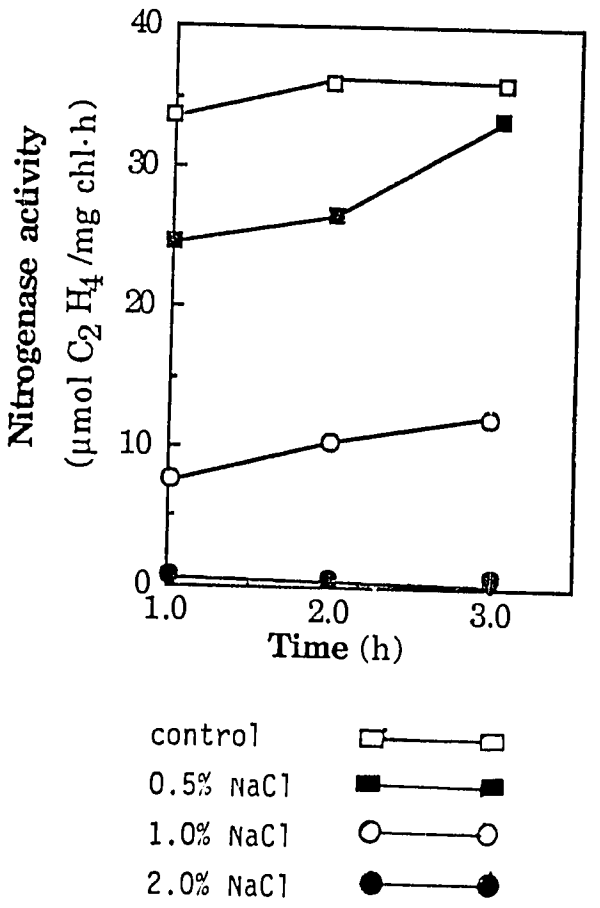
Fig 5. The effect of NaCl concentration on nitrogenase activity