

## BIOMASS, CHEMICAL COMPOSITION AND ABUNDANCE OF CYANOBACTERIA IN CYANOBACTERIAL INOCULA USED FOR APPLICATION IN RICE FIELDS

Herzalla, Nagat A.

Agricultural Microbiology Department, Soils, Water and Environment Research institute, Giza, Egypt

### ABSTRACT

A greenhouse experiment was conducted to evaluate the biomass, chemical composition and the abundance of cyanobacteria in the soil based cyanobacteria inocula (SBI) commonly used as biofertilizer in rice cultivation. Four local cyanobacteria strains viz. *Anabaena oryzae*, *Nostoc calcicola*, *Microchaete tenera* and *Cylindrospermum muscicola* were used each separately in addition to the control treatment (soil only without cyanobacteria inoculation) in the production of SBI inocula. Results show a large variability of biomass production of SBI depending on the incubation period. The indigenous cyanobacteria (control) gave its highest SBI biomass of 71.04 g m<sup>-2</sup> after one week then tended to decrease with increasing incubation time up to 4 weeks. Nitrogen percentages of the produced SBI ranged from 0.56 (control) to 2.00 (*C. muscicola*). Phosphorus Percentages ranged from 0.16 (*A.oryzae*) to 0.30 (*M.tenera* or *C.muscicola*). Carbon percentage ranged from 1.33 (control) to 7.87 (*N.calcicola*). The highest C/N ratio (8.86) noticed with *A. oryzae* after 4 weeks incubation period, while the lowest one (2.25) was for *C.muscicola* after 3 weeks incubation. The highest mean N/P ratio of 9.72 was for *C.muscicola* against the lowest one (4.18) for *A.oryzae*. The cyanobacteria count decreased with increasing the incubation up to 4 weeks.

### INTRODUCTION

Cyanobacteria constitute the largest, most diverse, and most widely distributed group of prokaryotes that performs oxygen photosynthesis. Several genera can fix atmospheric nitrogen (N<sub>2</sub>), and thus contribute to maintaining the fertility of natural and cultivated ecosystems, especially wet land rice fields (Roger and Kulasooriya 1980).

Cyanobacteria are the only N<sub>2</sub>-fixing microorganisms that generate their own photosynthate from CO<sub>2</sub> and water. This trophic independence has led many researchers to investigate their agronomic potential. Since De (1939) attributed the natural fertility of ricefields to cyanobacteria, hundreds of papers have been published on their use as bio fertilizer (Roger and Kulasooriya 1980 and Roger 1991).

Application of cyanobacteria inoculation into rice fields exerts a long lasting effect while mineral nitrogen like urea should be added more frequently. They are a promising alternative to avoid soil pollution caused by the use of agrochemicals, as well as recover the nutrient and structure lost after harvest (Mule *et al.*, 1999).

For a large scale application of cyanobacteria inoculation in rice cultivation, the production of the cyanobacterial inocula has been adopted long- ago in India, China, Viet Nam, Japan, Philippines (De, 1939; Watanabe,

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1966 and Venkataraman 1972). In Egypt, Ghazal (1987) studied the ecology of the soil based cyanobacteria inocula production to be applied in rice cultivation. He concluded that the two main considerations in the utilization of these cyanobacteria inocula in agriculture are (i) growing the cyanobacteria in abundance (mass culture), and (ii) preserving the produced cyanobacteria inocula for seeding purposes. However, despite the abundance of literature on the role of  $N_2$ -fixing cyanobacteria inocula and their possible use as a source of nitrogen for rice (Roger and Kulasooriya 1980), little is known about their chemical composition and the abundance of the cyanobacteria strains used in the production of these cyanobacteria inocula. Roger *et al.*, (1987) find out that the relative abundance of  $N_2$  fixing cyanobacteria in soil based inocula (SBI) ranged from 2 to 32 % and averaged 13-18 % with density number of the  $2.5 \times 10^8$  CFU g dry weight SBI<sup>-1</sup>. He also added that their chemical analyses ranged in average as 78-89 % ash, 2.1 %-4.7 % C, 0.2-0.8 % N, 640 – 1900 ppm P, N (0.5%), C (3.4%), and ash (80%) indicating that SBI inocula contain high percentage of soil. Applying the recommended dose of 10 kg SBI ha<sup>-1</sup> (Venkataraman, 1981) introduces  $2.5 \times 10^{11}$  CFU ha<sup>-1</sup> or  $2.5 \times 10^3$  CFU cm<sup>-2</sup>. This is 1/130<sup>th</sup> the average density of indigenous  $N_2$  fixing cyanobacteria ( $3.2 \times 10^5$  CFU cm<sup>-2</sup>) in the soils examined.

This paper is an attempt to evaluate (1) the cyanobacterial biomass of the produced soil based cyanobacteria inoculum (SBI), (2) its chemical composition, (3) the abundance of the cyanobacteria strains used in producing the (SBI) inoculum and (4) the suitable time for harvesting the produced cyanobacteria inoculum.

## MATERIALS AND METHODS

Slant agar refrigerated (5°C) cyanobacteria cultures were exposed to light (500 Lux) for 2 days then inoculated to liquid BG 11 (Rippika *et al.*, 1979) medium to reach the log phase growth of each alga strain. All cyanobacteria strains were occasionally purified using yeast extract agar medium. The developed algal growth was homogenized and then introduced as inoculum of (1ml) to 500 ml conical flasks containing 100 ml sterilized BG11 medium (pH 7.2) and incubated on continuous rotary shaking incubator (100 rpm) equipped with continuous illumination (3000 Lux) at temperature of 28 – 32°C. The developed cyanobacteria were then ready to use as inoculum starter for soil based cyanobacteria inoculum (SBI) production.

Shallow galvanized trays (65 cm x 65 cm x 15 cm) containing 5 kg of slightly alkaline clay loamy soil with pH 8.1, EC 2 dsm<sup>-2</sup>, organic matter 1.3 % (Allison 1965), total nitrogen 1.2 % (Jackson 1973) and available phosphorus 12.6 ppm Olsen (1972) were covered up to 10 cm height with tap water and supplied with 10g superphosphate (15 % P<sub>2</sub>O<sub>5</sub>), 12.5 ml sodium molybdate (in 1% solution, w/v) and 1.0 g carbofuran (ai 3 % granules) under the greenhouse condition to study the production of the soil based cyanobacteria inoculum (SBI) commonly used as biofertilizer in rice fields. The trays were inoculated with 50 ml inoculum starter from each of noitabuoni hcae fo dne eht

ssamoib lairetcabonayc fo sretemarap eht ,skeew ε dna τ ,γ ,δ fo sdoirep airetcabonayc dna oitar P/N ,N/C ,nobraç latot ,surohpsohp ,N – latot gUFC tinu rep demrof seinoloc<sup>-1</sup> soil were determined using the Plating technique of cyanobacteria enumerating (Allen and Stanier, 1968).

## RESULTS AND DISCUSSION

Results show a large variability of biomass production of SBI depending on the incubation period length. The indigenous cyanobacteria (control) gave its highest SBI biomass of 71.04 g m<sup>-2</sup> after one week then tended to decrease with increasing incubation time up to 4 weeks. Same trend was achieved for the inoculated cyanobacteria strains treatments that gave their corresponding highest biomass of 131.75, 100.11, 87.38 and 59.07 g SBI m<sup>-2</sup> after one week incubation period. Increasing time of incubation more than one week decreased significantly the biomass yield of SBI. The lowest SBI biomass of 37.39 g SBI m<sup>-2</sup> was recorded by *C. muscicola*.

Nitrogen percentages of the produced SBI ranged from 0.56 to 2.00. The highest percentage ratio of nitrogen (2.00) had achieved by *C. muscicola* at third week of incubation. While the least percentage nitrogen of 0.56 was for the control treatment after one week of incubation. However, the nitrogen percentage had averages of 0.81, 0.81, 1.25, 1.05 and 1.55 for control, *A. oryzae*, *N. calcicola*, *M. tenera* and *C. muscicola*, respectively.

In concern to phosphorus accumulation in the produced SBI inoculum, data in Table (1) and coincided that the incubation time elevation up to 4 weeks had decreased phosphorus percentages for both indigenous algae (control) and the inoculated ones. However, the respective mean threshold phosphorus percentages in harvested SBI inoculum were 0.16, 0.27, 0.30, 0.30 and 0.22 for control, *A. oryzae*, *N. calcicola*, *M. tenera* and *C. muscicola*, respectively. The highest phosphorus percentage (0.30) recorded by both *N. calcicola* and *M. tenera* against the lowest one (0.16) for the indigenous one (control).

Carbon percentages ranged from 1.33 (control) to 7.87 (*N. calcicola*). The mean values of carbon percentage were found to be in ascending of 3.67 (control), 5.05 (*A. oryzae*), 5.50 (*C. muscicola*), 5.62 (*N. calcicola*) and 5.83 (*M. tenera*). Carbon / nitrogen ratio ranged from 2.25 to 8.86. The highest C/N ratio (8.86) noticed with *A. oryzae* after 4 weeks incubation period, while the lowest one (2.25) was for *C. muscicola* after 3 weeks incubation. Nevertheless, the C/N ratios could be arranged according to their mean values as 3.72 (*C. muscicola*), 5.90 (*A. oryzae*). It is also obvious that C/N ratio increased gradually with increasing incubation time up to the third week in parallel with produced algal biomass.

In contrast to the decrease of phosphorus with increasing time of production, it was detected that N/P ratio increased with raising time of SBI inoculum production with average ratios of 5.57, 4.18, 5.98, 5.27 and 9.72 for control, *A. oryzae*, *N. calcicola*, *M. tenera* and *C. muscicola*, respectively. However, the highest mean N/P ratio of 9.72 was for *C. muscicola* against the lowest one (4.18) for *A. oryzae*. The nitrogen phosphorus ratio seemed to be

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Table (1): Biomass and chemical composition of produced cyanobacterial soil based inoculum

Treatment algae strains	control	<i>A. oryzae</i>	<i>N. calcicola</i>	<i>M. tenera</i>	<i>C. muscicola</i>
<u>Incubation time week</u>			<u>Mass culture g / m<sup>2</sup></u>		
1	71.04	131.75	100.11	87.38	59.07
2	67.08	74.47	57.77	53.98	57.61
3	56.12	60.04	44.61	45.67	54.02
4	45.28	43.24	40.00	39.32	37.39
			<u>Nitrogen percentages</u>		
1	0.56	0.57	0.79	0.71	0.78
2	0.90	0.93	1.26	0.93	1.83
3	0.86	0.89	1.35	1.38	2.33
4	0.90	0.85	1.58	1.19	1.60
			<u>Phosphorus percentages</u>		
1	0.20	0.40	0.62	0.42	0.26
2	0.19	0.40	0.37	0.31	0.20
3	0.15	0.17	0.16	0.26	0.23
4	0.10	0.11	0.15	0.12	0.17
			<u>Carbon percentages</u>		
1	1.33	2.77	3.27	3.48	4.17
2	4.97	4.83	5.03	5.37	7.57
3	4.26	5.05	7.87	6.50	5.24
4	4.10	7.53	6.29	7.98	5.00
			<u>C / N ratio</u>		
1	2.38	4.86	4.14	4.90	5.36
2	5.52	5.19	3.99	5.77	4.14
3	4.95	5.67	5.83	4.71	2.25
4	4.56	8.86	3.98	6.71	3.13
			<u>N / P ratio</u>		
1	2.80	1.43	1.52	1.69	3.00
2	4.74	2.33	3.41	3.00	9.15
3	5.73	5.24	8.44	5.31	10.13
4	9.00	7.73	10.53	7.44	10.00

L. S. D. values for the a forementioned parameters:

	Biomass		N	
	0.05	0.01	0.05	0.01
A	2.46	3.30	0.27	0.36
B	2.75	3.69	0.30	0.41
AB	1.23	1.65	0.14	0.18
	P		C	
	0.05	0.01	0.05	0.01
A	0.09	0.12	1.23	1.65
B	0.10	0.13	1.38	1.85
AB	0.04	0.06	0.62	0.83

A, Strain; B, period;

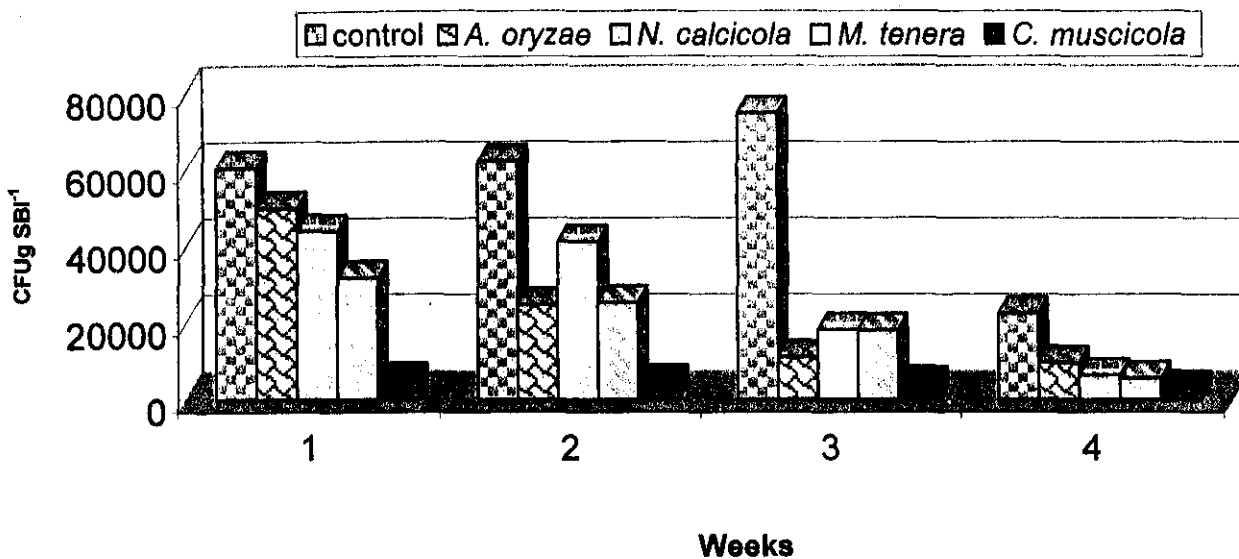


Fig.(1): Occurrence of cyanobacteria in produced soil based algae inoculum

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in reverse relation with the produced algal biomass either for the control treatment or the other tested strains.

Total cyanobacteria count was evaluated in the produced SBI at intervals of 1, 2, 3 and 4 weeks (Fig. 1) by plating dilution technique (dry weight basis) on agarized BG 11 medium (Allen and Stanier 1968). The total cyanobacteria count in control treatment had increased with increasing the incubation period up to 3 weeks, then started to decrease at the fourth weeks. The corresponding total population counts were 60730, 62400, 75060 and 22530 CFU g SBI<sup>-1</sup> in respective to 1, 2, 3 and 4 weeks. In the contrary with the control treatment, the population count of other cyanobacteria had been decreased with rising the incubation period up to 4 weeks. The corresponding total count in respective to incubation period of 1, 2, 3 and 4 weeks were 50100, 24800, 11100 and 9730 CFU g SBI<sup>-1</sup> (*A. oryzae*), 44060, 41300, 18300 and 6460 CFU g SBI<sup>-1</sup> (*N. calcicola*), 32000, 25400, 18160 and 5630 CFU g SBI<sup>-1</sup> (*M. tenera*) and 5400, 4660, 4260 and 3200 CFU g SBI<sup>-1</sup> (*C. muscicola*). Among the tested cyanobacteria strains, *A. oryzae* recorded the highest occurred population of 50100 CFU g SBI<sup>-1</sup> at the first incubation week followed with respective population of 44060, 32000 and 5400 CFU g SBI<sup>-1</sup> corresponding to *N. calcicola*, *M. tenera* and *C. muscicola*. The lowest population occurrence had achieved by *C. muscicola* (3200 CFU g SBI<sup>-1</sup>) at the fourth incubation week.

However, it is obvious that to prepare the SBI biofertilizer, it is better to harvest the cyanobacteria mat produced after one week of cyanobacteria cultivation. This trend was to avoid the competition of the indigenous algae that inhabitant naturally the soil and which overcome the survival of the inoculated cyanobacteria with prolonged incubation time more than one week (Fig. 1).

The rice field ecosystem provides a favorable environment for the cyanobacteria with respect to their requirements for light, water, high temperature and nutrient availability. This may account for higher abundance of cyanobacteria in paddy soils than in other cultivated soils (Watanabe and Yamamoto, 1971). Therefore it was useful to save such conditions under the Egyptian condition to may produce high quality cyanobacterial inoculum to accelerate nitrogen saving for rice production.

## REFERENCES

- Allen, M. M. and R. Y. Stanier (1968): Selective isolation of blue-green algae from water and soil. *J. Gen. Microbiol.* 51: 203 – 209.
- Allison, L.E. (1965). Walkely Black method. In: C.A. Black (Ed.), *Methods of soil Analysis*, Part 2: 1372 – 1376. Amer. Soc. Agron., Madison, Wisc
- De, P. K. (1939). The role of blue-green algae in nitrogen fixation in rice fields. *Proc. R. Soc. Lond.* 127 B: 121-139.
- Ghazal, F. M. (1987). Microbiological studies on nitrogen fixation by alga and *Azolla*. Ph.D. Thesis, Fac. Agric. Al-Azhar Univ., Cairo, Egypt.
- Jackson, M. L. (1973). *Soil chemical analysis*. Constable Co. Lt. London.

- Mule, M. C. Z., Caire, G. Z., Cano, M. S., Palma, M. R. and Karina Colombo. (1999). Effect of cyanobacterial inoculation and fertilizers on rice seedlings and postharvest soil structure. *Commun. Soil, Sci. Plant Anal.*, 30: 97-107.
- Olsen, G. (1972). On biological nitrogen fixation in nature, particular in blue-green algae. *C.R. Trav. Lab. Carlsberg*. 37 : 269 – 283.
- Rippika, R.; J. Deruelles; J. B. Waterbury; M Herdman, and R. Y. Stanier. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria *J. Gen. Microbiol.* 111 : 1 – 61.
- Roger, P. A. (1991). Reconsidering the utilization of blue-green algae in wetland rice cultivation. Pages 119-141 in *Biological N2 fixation associated with rice production*. S. K. Dutta and C. Sloger, eds. Oxford and IBH Publishing, New Delhi.
- Roger, P. A. and S. A. Kulasooriya (1980). Blue-green algae and rice. *Edt. International Rice Res. Inst.*, pp.51-52.
- Roger, P. A. S. Santiago-Ardales, P. M. Reddy and I. Watanabe. (1987). The abundance of heterocystous blue-green algae in rice soils and inocula used for application in rice fields. *Biol. Ferti. Soils*, 5: 98 – 105.
- Venkataraman, G. S. (1981). Blue-green algae for rice production. A manual for its promotion. *FAO Soil Bull.* no. 46.
- Venkataraman, G. S. (1972). *Biofertilizer and rice cultivation. Today and Tomorrow*, New Delhi, P.81.
- Watanabe, A. (1966). The blue-green algae as the nitrogen fixators. *Proc. IX Int. cong. Microbiol., Moscow*. 77-85.
- Watanabe, A. And Y. Yamamoto. (1971). Algal nitrogen fixation in the tropics. *Plant and Soil, special vol.* 403-413.

### الكتلة الحية والتركيب الكيميائي وكذلك خلايا السياتو بكتريا الموجودة فى لقاح

#### السيانوبكتريا المنتج باستخدام التربة

نجاه عبد العزيز حرزالله

قسم الميكروبيولوجيا الزراعية – معهد بحوث الأراضى والمياه والبيئة – الجيزة- مصر

فى هذه الدراسة أجريت تجربة فى الصوبة باستخدام أربعة سلالات من السياتوبكتريا كل على حدة وكانت هذه السلالات هي:

*Anabaena oryzae*, *Nostoc calcicola*, *Microshaete tenera*, and  
*Cylindrospermum muscicola*

وذلك لتقييم لقاح السياتوبكتريا المنتج باستخدام التربة وذلك من حيث الكتلة المنتجة والتركيب الكيميائي له وكذلك أعداد خلايا السياتوبكتريا لكل جرام جاف منتج من هذا اللقاح . ولقد أوضحت النتائج ما يلى:

- ١- لقد كان هناك اختلاف فى وزن الكتلة الحية من اللقاح الناتج وذلك باختلاف فترة التحضين .
- ٢- أعطت معاملة المقارنة (الكنترول) أعلى وزن جاف لها بعد أسبوع من النمو ثم انخفضت هذه الكمية بعد ذلك وحتى الأسبوع الرابع .
- ٣- تراوحت النسبة المئوية للنتروجين باللقاح الناتج بين ٥٦% و ٢% .
- ٤- تراوحت النسبة المئوية للفوسفور باللقاح الناتج بين ١٦% و ٣٠% .
- ٥- تراوحت النسبة المئوية للكربون باللقاح الناتج بين ١٠٣٣% و ٧٠٨٧% .
- ٦- لقد تحققت أعلى نسبة من الكربون الى النتروجين (٨٠٨٦) بواسطة سلالة *A. oryzae* .
- ٧- لقد تحققت أعلى نسبة من النتروجين الى الفوسفور (٩٠٧٢) بواسطة سلالة *C. muscicola* .