Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP4: 68-71

Ruby Chandra
University Dept. of Botany,
J. P. University, Chapra, Bihar,
India

Pawan Kumar Srivastava
University Dept. of Botany,
J. P. University, Chapra, Bihar,
India

Kanhaiyaji Verma
University Dept. of Botany,
J. P. University, Chapra, Bihar,
India

Correspondence
Ruby Chandra
University Dept. of Botany,
J. P. University, Chapra, Bihar,
India

(Special Issue- 4)
International Conference on Food Security and Sustainable Agriculture
(Thailand on 21-24 December, 2018)

Effect of some nutrients on growth of rice field cyanobacteria

Ruby Chandra, Pawan Kumar Srivastava and Kanhaiyaji Verma

Abstract

Rice fields situated north of Railway Station of Chapra were surveyed for the occurrence of heterocystous cyanobacterial strains. Two cyanobacterial strains namely *N. linckia* and *A. doliolum* were isolated and selected for further studies. Effect of some nutrients on growth of the two strains was evaluated in different culture media. Since the selected strains are heterocystous and nitrogen fixing, elemental nitrogen (N_2) as well as combined inorganic nitrogen (NO_3) media were used.

Such studies might be helpful in deciding the use of fertilizers in rice fields along with cyanobacteria as biological nitrogen fixers. On the basis of results the quality and quantity of fertilizers to be used in rice fields may be decided so that the God gifted N-fertilizer may not be adversely affected in farmers field.

Keywords: Heterocyst, nitrogen-fixation, nitrogen, phosphate, potassium

Introduction

Rice field cyanobacteria are exposed to various agrochemicals in form of fertilizers and pesticides. Since these microorganisms are useful for the rice fields so far as its nitrogen economy is concerned. They are helpful in increasing the utilizable form of nitrogen by way of biological nitrogen fixation (Singh, P.K. and Bisoyi, R. N. 1989: Kaushik, 2008; Dey, *et al.*, 2010; Choudhary, 2011) [12. 4, 3] It is thus desirable to know the effect of some important chemicals on the growth of the selected heterocystous forms of cyanobacteria thriving in the rice fields of Chapra.

Materials and Methods

A suitable culture medium should be decided for the selected strains and then the concentration of some of the important constituent are altered for obtaining better growth under prevailing laboratory conditions. pH of culture medium is one of the important factor for the growth of any microorganism. N/10 NaOH and bench HCl was used to adjust the ph of the culture medium to 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0. 10 ml of each such medium was inoculated with equal amount of nitrate grown culture. After 24 days of incubation in culture cabinet growth was recorded in terms of optical density of the pigments extracted from the exponentially grown culture. Out of the different constituents of the selected culture medium, Nitrogen, Phosphorous, Calcium and Potassium were selected for the present study. Different levels and source of these nutrients were used for determining their optimum utilization by the selected cyanobacterial strains. All such experiments were conducted in both elemental N_2 as well as combined inorganic nitrogen (NO_3) medium.

Result

Four culture media (Chu No. 10, Chu No 10 as modified by Gerloff *et al.*, 1950, Fogg: 1949, and Allan & Arnon 1950) were used to find out the most suitable one for growth of selected cyanobacterial strains. The results are given in table 1

Table 1: Growth of the cultures in different culture media

Selected strains	Without combined N_2 (N_2-free medium)			
	Chu No.10 (1942)	**Mod. Chu No.10(1950)**	**Fogg (1949)**	**Allen & Arnon(1950)**
N. linckia	0.48 ± 0.012	0.52 ± 0.012	0.44 ± 0.011	0.40 ± 0.013
A. doliolum	0.50 ± 0.009	0.58 ± 0.014	0.44 ± 0.013	0.42 ± 0.012
Combined Nitrogen (NO_3^-) medium				
N. linckia	0.52 ± 0.011	0.64 ± 0.012	0.48 ± 0.011	0.46 ± 0.012
A. doliolum	0.52 ± 0.012	0.62 ± 0.013	0.50 ± 0.013	0.44 ± 0.011

Effect of pH on growth
pH is one of the main factors which influences the growth of cyanobacteria in rice fields. Slightly alkaline pH is preferred by the two algae. However, *N. linckia* grew well at 7.0 pH whereas *A. doliolum* appeared to prefer slightly higher pH 7.5. No variation in requirement of pH was observed in N_2 or NO_3^- media. The results are discussed in table 2.

Table 2: Growth of the two algae at different levels of pH in N_2 & NO_3^- culture media

pH	*N.linckia*		*A.doliolum*	
	N_2 – medium	**NO_3^-–medium**	**N_2 -medium**	**NO_3^--medium**
6	0.30 ± 0.014	0.34 ± 0.011	0.29 ± 0.018	0.34 ± 0.014
6.5	0.32 ± 0.012	0.36 ± 0.012	0.32 ± 0.014	0.39 ± 0.013
7	0.46 ± 0.015	0.48 ± 0.014	0.32 ± 0.013	0.48 ± 0.014
7.5	0.44 ± 0.011	0.47 ± 0.013	0.48 ± 0.014	0.54 ± 0.016
8	0.44 ± 0.012	0.46 ± 0.011	0.46 ± 0.012	0.52 ± 0.018
8.5	0.44 ± 0.014	0.44 ± 0.014	0.46 ± 0.011	0.49 ± 0.013
9	0.40 ± 0.013	0.42 ± 0.015	0.44 ± 0.016	0.45 ± 0.012
9.5	0.38 ± 0.012	0.40 ± 0.013	0.44 ± 0.011	0.45 ± 0.011
10	0.34 ± 0.011	0.40 ± 0.012	0.41 ± 0.011	0.41 ± 0.012
10.5	0.32 ± 0.013	0.38 ± 0.009	0.40 ± 0.014	0.40 ± 0.011

Effect of various source and concentration of selected nutrients on growth
Nitrogen

Three different combined source of nitrogen (KNO_3, KNO_2 and NH_4Cl) were used at different concentrations to ascertain the most suitable source of nitrogen. Growth was recorded on 24th day of inoculation as shown in table 3.

Table 3: Growth and heterocyst frequency of *N. linckia* and *A. doliolum* at different levels and sources of nitrogen

Source	Level of N_2	*N. linckia*		*A. doliolum*	
		Growth	**Het. Freq.**	**Growth**	**Het. Freq**
KNO_3	3.4	0.37 ± 0.011	4.5 ± 0.32	0.36 ±0.018	3.8 ±0.24
	6.8	0.38 ± 0.012	5.1 ± 0.28	0.37 ± 0.011	3.5 ± 0.19
	13.6	0.44 ± 0.013	4.3± 0.26	0.43 ± 0.012	2.2 ± 0.18
	34	0.52 ± 0.016	1.6 ± 0.28	0.44 ± 0.013	0
	68	0.54 ± 0.016	0	0.48 ± 0.014	0
	102	0.42 ± 0.011	0	0.46 ± 0.016	0
	134	0.38 ± 0.014	0	0.38 ± 0.13	0
KNO_2	3.4	0.38 ± 0.013	4.42 ± 0.26	0.38 ± 0.016	3.51 ± 0.32
	6.8	0.41 ± 0.012	3.71± 0.25	0.37 ± 0.018	2.61 ± 0.35
	13.6	0.46 ± 0.015	2.81±0.21	0.43 ± 0.015	0
	34	0.49 ± 0.013	0	0.50 ± 0.016	0
	68	0.50 ± 0.011	0	0.52 ± 0.011	0
	102	0.48 ± 0.009	0	0.46 ± 0.014	0
	134	0.46 ± 0.012	0	0.43 ± 0.012	0
NH_4Cl	3.4	0.40 ± 0.012	0	0.38 ± 0.012	2.00 ± 0.28
	6.8	0.44 ± 0.004	2.01± 0.18	0.42 ± 0.012	1.41 ± 0.12
	13.6	0.48 ± 0.015	0	0.46 ± 0.016	0
	34	0.58 ± 0.009	0	0.52 ± 0.012	0
	68	0.55 ± 0.014	0	0.50 ± 0.018	0
	102	0.51 ± 0.016	0	0.48 ± 0.019	0
	134	0.48 ± 0.012	0	0.39 ± 0.012	0
Elemental Nitrogen		0.34 ± 0.001	5.8± 0.33	0.34 ± 0.21	4.5 ± 0.38

Phosphate

Results of the above experiments have been summarized in Table-4. It is evident from the result that phosphate is required more in elemental nitrogen medium than nitrate medium and K_2HPO_4 appears to have been preferred than Na_2HPO_4 by both the strains.

Table 4: Effect of phosphate on growth in N_2 & NO_3^- medium

Source of PO_4	Concen. of Phosphate (ppm)	*N. linckia*		*A. doliolum*	
		N_2 Medium	NO_3^- medium	N_2 Medium	NO_3^- Medium
K_2HPO_4	0	0.24 ± 0.016	0.25 ± 0.014	0.22± 0.012	0.24 ± 0.019
	1.34	0.31 ± 0.012	0.34 ± 0.012	0.28± 0.011	0.36 ± 0.013
	2.68	0.40 ± 0.011	0.46 ± 0.015	0.32± 0.012	0.42 ± 0.014
	5.37	0.46 ± 0.012	0.58 ± 0.012	0.41± 0.015	0.56 ± 0.015
	10.73	0.50 ± 0.015	0.54 ± 0.011	0.48± 0.012	0.55 ± 0.013
	16.10	0.47 ± 0.013	0.52 ± 0.015	0.44± 0.013	0.49 ± 0.019
	26.83	0.45 ± 0.015	0.50± 0.014	0.35± 0.012	0.46 ± 0.015
	53.66	0.40 ± 0.013	0.49 ± 0.013	0.32± 0.018	0.44 ± 0.012
Na_2HPO_4	1.34	0.28 ± 0.012	0.25 ± 0.015	0.28± 0.011	0.22 ± 0.013
	2.68	0.38 ± 0.011	0.45 ± 0.012	0.30± 0.017	0.32 ± 0.016
	5.37	0.44 ± 0.014	0.58 ± 0.014	0.39 ± 0.016	0.38 ± 0.014
	10.73	0.48 ± 0.013	0.47 ± 0.013	0.44 ± 0.014	0.49 ± 0.011
	16.10	0.45 ± 0.015	0.44 ± 0.012	0.38 ± 0.012	0.54 ± 0.019
	26.83	0.43 ± 0.012	0.42 ± 0.011	0.34 ± 0.012	0.50 ± 0.015
	53.66	0.35 ± 0.011	0.37 ± 0.013	0.31 ± 0.014	0.41 ± 0.014

Potassium

Results have been summarized in Table 5 which indicates that the two algae do not show any difference in the requirement of potassium for their growth. However, requirement of potassium is more in N_2 medium than in NO_3^- medium.

Table 5: Effect of potassium on growth in N_2 and NO_3 medium

Concen. of Potassium (K) Ppm	*N. linckia*		*A. doliolum*	
	N_2 Medium	NO_3^- medium	N_2 Medium	NO_3^- Medium
0	0.24 ± 0.012	0.28 ± 0.014	0.22 ± 0.011	00.26 ± 0.013
1.14	0.36 ± 0.014	0.37 ± 0.012	0.34 ± 0.015	0.37 ± 0.014
2.28	0.38 ± 0.016	0.40 ± 0.015	0.39 ± 0.016	0.44 ± 0.013
4.57	0.40 ± 0.012	0.46 ± 0.013	0.42 ± 0.013	0.50 ± 0.014
9.14	0.46 ± 0.013	0.44 ± 0.015	0.46 ± 0.012	0.48 ± 0.012
114.2	0.44 ± 0.012	0.42 ± 0.011	0.42 ± 0.015	0.44 ± 0.013
22.84	0.42 ± 0.011	0.42 ± 0.016	0.40 ± 0.011	0.46 ± 0.012

Discussion

The abiotic factors such as pH, light, salinity, temperature and nutrient responses are useful to establish the appropriate conditions for optimizing cyanobacterial and microalgal growth (Harrison *et al*., 1993; Rocha *et al*., 2003; Bano and Siddiqui, 2004; Raghavan *et al*., 2008) [5, 8, 1, 7]. Rice fields are generally alkaline in nature and are natural habitat of N_2-fixing cyanobacteria. Rice fields of Chapra (North of Chapra Railway station) have been studied for the occurrence of nitrogen fixing cyanobacteria. The two selected strains are filamentous and heterocystous. Heterocysts are the site if nitrogen fixation which provides anaerobic condition for the activity of the enzyme, nitrogenase (Wolk, 1968; Tel-Or and Stewart, 1976, Stewart, 1980) [14, 13, 10]. There is two heterocystous strains grew well in the absence of combined inorganic source of nitrogen i.e. N_2-medium. Ammoniacal nitrogen (NH_4Cl) is utilized better than the other source of combined nitrogen (Nitrite and Nitrate). On the basis of similar results Guerrero and Cataline, 1987 has suggested that there are some regulatory interactions between the involved metabolic processes. Higher level of ammoniacal nitrogen adversely affects the growth as compared to the nitrite and nitrate nitrogen. Singh, 1985 and 1989 [11, 12]; Roger and Kulasooriya, 1980 have noticed reduced growth of cyanobacteria in the presence of N-fertilizers in paddy fields. Some growth has been observed in potassium deficient culture. Therefore, the requirement of potassium is not absolute for the growth of the test materials. No increase in biomass production of cyanobacteria has been found in the rice fields where potassium fertilizers are used (Singh and Bisoyi, 1989) [12]. Similar results have been reported by Wilson and Alexander (1979) [15]. Phosphorus has been found to be one of the essential nutrients for the growth of the algae (Roger *et al*., 1987; Bisoyi and Singh, 1988a) [9, 2]. It is evident from the results that K_2HPO_4 source of phosphate is preferred in comparison to Na_2HPO_4. On the basis of above results the quality and quantity of fertilizers to be used in rice fields may be decided so that the God gifted N-fertilizer may not be adversely affected.

Reference

1. Bano A, Siddiqui PJA. Characterization of five marine cyanobacterial species with respect to their pH and salinity requirements. Pak. J Bot. 2004; 36:133-143.
2. Bisoyi RN, Singh PK. Effect of seasonal changes on cyanobacterial production and nitrogen yield. Microbial. Eco. 1988a; 16:149-154.
3. Choudhary Kaushal Kishore. Occurrence of Nitrogen fixing Cyanobacteria during different stages of paddy cultivation. Bangladesh J Plant Taxon. 2011; 18(1):73-76.
4. Dey HS, Tayung K, Bastia AK. Occurrence of nitrogen Fixing cyanobacteria in local rice fields of Orissa, India, Ecoprint, Ecological Society (ECOS), Nepal. 2010; 17:77-85,
5. Harrison PJ, Thompson PA, Guo M, Taylor FJR. Effects of light, temperature and salinity on the growth rate of harmful marine diatoms, Chaetoceros convolutus and C. concavicornis that kill netpen salmon. J Appl. Phycol. 1993; 5:259-265.
6. Kaushik BD, Prasana R. Improved cyanobacterial Biofertilizer production and N–saving in rice cultivation In: suitable aquaculture. (eds) Sahoo, D and S. Z. Quasin. P. PH. publishing corporation, New Delhi, 2002, 145-

155.
7. Raghavan G, Haridev CK, Gopinathan CP. Growth and proximate composition of the Chaetoceros calcitrans f. pumilus under different temperature, salinity and carbon dioxide levels. Aquacult. Res. 2008; 39:1053-1058.
8. Rocha JM, Garcia JE, Henriques MH. Growth aspects of the marine microalga Nannochloropsis gaditana. Biomol. Eng. 2003; 20:237-242.
9. Roger PA. The abundance of heterocystous blue-green algae in rice Soils and inocula used for application in rice fields. Biol. Fert. Soil. 1987; 5:98-105.
10. Stewart WDP. Some aspects of structure and function in N_2 – fixing cyanobacteria. Ann. Rev. Microbiol. 1980; 34:497-536.
11. Singh PK. Nitrogen fixation by the blue green algae in paddy fields. In Rice Research in India, ICAR, New Delhi, 1985, 344-362.
12. Singh PK, Bisoyi RN. Blue green algae in rice fields. Phykos. 1989; 28(182):181-195.
13. Tel-Or E, Stewart WDP. Photosynthetic electron transport, ATP synthesis and nitrogenise activity in isolated heterocyst of *Anabaena cylindrica*. Proc. Roy. soc. Lond. 1976; 198:61-86.
14. Wolk CP. Movement of carbon from vegetative cells to heterocysts in *Anabaena cylindrical*. J Bacteriol. 1968; 96:2138-2143.
15. Wilson JT, Alaxander M. Effect 0f soil nutrient status and pH on nitrogen fixing algae on flooded soils. Social Sci. Amer. J. 1979; 43:936-939.