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RESPONSE OF *ANABAENA* SPECIES TO DIFFERENT NITROGEN SOURCES

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Nitrogenase activity, ammonia excretion and glutamine synthetase (GS) activity were examined in five strains of *Anabaena* (*A. anomala* ARM 314, *A. fertilissima* ARM 742*, A. variabilis* ARM 310, *A. oryzae* ARM 313 and *A. oryzae* ARM 570) in the presence of 2.5 mM NO_3-N (KNO_3), 2.5 mM NH_4^--N [$(NH_4)_2SO_4$] and diatomic nitrogen (N_2). Ammonium-N was more inhibitory to nitrogenase activity as compared to NO_3-N in all the strains. Maximum GS activity was exhibited in NO_3-N medium, irrespective of the cyanobacterial strains studied. Uninduced release of ammonia was observed in all the species, with *A. oryzae* ARM 313 and *Anabaena variabilis* ARM 310 exhibiting maximum excretion of 0.25–0.31 and 0.27–1.23 μ moles NH_4^- mg Chl^{-1} respectively on the 15th day of incubation. The glutamine synthetase activity of *A. oryzae* ARM 313 was relatively very high as compared to *Anabaena variabilis* ARM 310. There was no nitrate reductase activity in any of the *Anabaena* sp. grown on NH_3-N or N_2-N on the 15th day of incubation.

Keywords: Cyanobacteria – nitrogenase – GS activity – ammonia excretion – *Anabaena*

INTRODUCTION

Cyanobacteria are lower photosynthetic procaryotes. Some of the species have terminally differentiated structures called heterocysts. These structures are known to be involved in fixation of atmospheric nitrogen [10]. However, some non-heterocystous species are also reported to fix atmospheric nitrogen [22, 39]. Apart from fixation and utilization of atmospheric nitrogen, cyanobacteria are capable of utilizing NO_3-N [2], NO_2-N [20] and ammonium-N [1]. Although cyanobacteria are known to be one of the promising supplements to nitrogenous fertilizer, the process of biological nitrogen fixation, mediated through the enzyme nitrogenase, may be inhibited in the presence of readily available nitrogen sources. This inhibition may be either at the level of heterocyst differentiation or the synthesis of enzyme nitrogenase. It has been shown that the nitrogenase activity and synthesis are inhibited by NO_3-N [35] and ammonium-N [11, 27]. But contrary reports on high nitrogenase activity in the pres-

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ence of high levels of ammonium-N as in case of *Anabaena flos-aquae* [3], *Anabaena cylindrica* [21] and *Anabaena* sp. strain CA [4] also exist. Hence, cyanobacterial strains capable of fixing nitrogen in presence of readily available sources of nitrogen have immense value in cyanobacterial biofertilizer production programme.

The key enzyme involved in assimilation of nitrogen is glutamine synthetase [36]. Therefore, for the efficient utilization of cyanobacteria in biofertilizer inoculum production, strains showing rapid growth, high nitrogenase activity, low/deficient GS activity/ammonia transport system leading to increased excretion of ammonia are most relevant [23].

One of the diazotrophic genera observed commonly in all types of paddy soils is *Anabaena* which is used in cyanobacterial biofertilizer production. Therefore, the investigation was directed towards studying the effect of various N-sources on different species of *Anabaena* and selecting a strain capable of maximizing the process of fixation and excretion of fixed nitrogen.

MATERIAL AND METHODS

Screening and selection of species

From a large number of *Anabaena* species available at National Centre for Conservation & Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi, five strains were selected. The selection was primarily based on faster growth rate, high nitrogenase activity and ammonia excretion. These were *Anabaena variabilis* ARM 310, *A. anomala* ARM 314, *A. oryzae* ARM 313, *A. oryzae* ARM 570 and *A. fertilissima* ARM 742.

Growth conditions

Anabaena species were grown under continuous illumination (2500 lux, white light) at 29 ± 1 °C in nitrogen free BG-11 medium [32]. The experiments involving exogenous nitrogen sources were supplemented with either 2.5 mM KNO_3 or 2.5 mM $(NH_4)_2SO_4$ as per requirement. NH_4^+-N medium was buffered with MOPS buffer. Growth was measured as chlorophyll [19] or total protein content [12].

Enzyme assays

Glutamine synthetase (GS) activity was measured by the method of Shapiro and Stadtman [28] as modified by Stacey et al. [31]. The release of ammonia in the medium was estimated by the method of Solorzano [30]. Nitrogen fixing potential was measured using acetylene reduction activity (ARA) as an index of nitrogenase activ-

ity or nitrogen fixation [14], using Gas chromatograph with flame ionization detector and nitrogen as carrier gas. Nitrate reductase (NR) activity was estimated by measuring the nitrite following the method of Lowe and Evans [17].

RESULTS

Growth studies

Growth of the different strains presented as growth rate (GR) was measured as chlorophyll and protein (Table 1). It showed an increasing trend with time of incubation, with the maximum being achieved at 15th day of incubation. Among the different nitrogen sources, maximum chlorophyll was observed in KNO_3 supplemented medium for ARM 313, ARM 570 and ARM 742, while ARM 314 showed best growth on elemental nitrogen. ARM 310 was the only strain which showed maximum growth in medium supplemented with $(NH_4)_2SO_4$, the growth of this strain on KNO_3 or N_2 was less than on ammonium-N.

As chlorophyll, protein synthesis in these strains also showed a similar increasing trend with time. The nature of nitrogen source was observed to contribute to the differences in the total protein synthesized by the various strains. The protein content was maximum in ARM 310 grown in ammonium-N medium, followed by that grown in N_2-N and KNO_3 medium. The protein synthesis was stimulated in presence of KNO_3-N in ARM 313, whereas $(NH_4)_2SO_4$-N was favourable for ARM 570 (Table 1).

Table 1
Growth rate day^{-1} measured as chlorophyll µg ml^{-1} and protein mg ml^{-1} in response to different nitrogen sources up to 15 days of growth cycle

Anabaena sp. & ARM No.	Nitrogen sources					
	N≡N		KNO_3		$(NH_4)_2SO_4$	
	Chl	protein	Chl	protein	Chl	protein
A. oryzae 313	0.39	0.07	0.55	0.09	0.14	0.08
A. anomala 314	0.64	0.04	0.54	0.04	0.11	0.03
A. oryzae 570	0.56	0.08	0.78	0.08	0.11	0.12
A. variabilis 310	0.32	0.30	0.28	0.27	0.51	0.40
A. fertilissima 742	0.20	0.04	0.24	0.05	0.11	0.04

C.D at 5%	As Chl	As protein
1. Among mean of strains (S)	0.024	0.013
2. Among nitrogen sources (N)	0.026	0.031
3. Interactions S × N	0.034	0.036

Chl – Chlorophyll

Table 2
Effect of different nitrogen sources on the heterocyst frequency (%) and nitrogenase activity measured as acetylene reduction activity (ARA) (μ moles C_2H_4 mg^{-1} Chlorophyll hr^{-1}) of *Anabaena* sp.

Organisms	Time in days	N-sources					
		Control N≡N		KNO_3		$(NH_4)_2SO_4$	
		Heterocyst	N_2ase activity	Heterocyst	N_2ase activity	Heterocyst	N_2ase activity
A. oryzae ARM 313	0	6.50	0.42	6.50	0.42	6.50	0.42
	4	3.80	0.87	2.87	0.41	1.60	0.35
	7	4.10	1.28	2.29	0.32	0.06	0.24
	10	5.55	3.62	1.92	0.20	0	0
	15	5.83	0.89	1.54	0.13	0	0
Het. vs. ARA (r)		0.0101		0.7281		0.7557	
A. anomala ARM 314	0	4.57	0.38	4.57	0.38	4.57	0.38
	4	3.45	0.52	1.25	0.30	0.05	0.06
	7	3.65	0.86	1.04	0.28	0	0.03
	10	4.26	2.96	0.19	0.25	0	0.01
	15	4.82	0.82	0.16	0.10	0	0
Het. vs. ARA (r)		0.0793		0.1805		0.9910	
A. oryzae ARM 570	0	5.84	0.56	5.84	0.56	5.84	0.56
	4	3.82	0.98	2.88	0.48	1.68	0.38
	7	4.16	1.43	2.31	0.41	0.06	0.27
	10	5.65	3.77	1.94	0.38	0	0.06
	15	5.87	1.01	1.56	0.33	0	0
Het. vs. ARA (r)		0.1798		0.9352		0.8612	

Table 2 (cont.)

Organisms	Time in days	N-sources					
		Control N≡N		KNO_3		$(NH_4)_2SO_4$	
		Heterocyst	N_2ase activity	Heterocyst	N_2ase activity	Heterocyst	N_2ase activity
A. variabilis ARM 310	0	3.90	0.32	3.90	0.32	3.90	0.32
	4	3.07	0.49	2.12	0.30	1.30	0.21
	7	3.50	0.77	1.10	0.23	0.05	0.13
	10	3.85	2.00	1.01	0.16	0	0
	15	3.96	0.63	0	0.01	0	0
Het. vs. ARA (r)		0.2508		0.8276		0.8952	
A. fertilissima ARM 742	0	3.17	0.31	3.17	0.31	3.17	0.31
	4	3.53	0.42	2.39	0.30	1.61	0.19
	7	3.55	0.70	2.28	0.20	0.07	0.06
	10	3.66	1.98	1.89	0.12	0	0.02
	15	3.84	0.78	0.12	0.07	0	0
Het. vs. ARA (r)		0.4852		0.8712		0.9942	

r – Correlation coefficient
Het. – heterocyst frequency

Table 3
Effect of different nitrogen sources on glutamine synthetase (GS) activity (μmol γ-glutamyl hydroxamate mg^{-1} protein) and ammonia excretion (AE) (μ moles mg^{-1} Chlorophyll) of *Anabaena* sp.

Organisms	Time in days	N-sources					
		Control N≡N		KNO_3		$(NH_4)_2SO_4$	
		GS	AE	GS	AE	GS	AE
A. oryzae ARM 313	0	8.93	1.33	8.93	1.33	8.93	1.33
	4	24.15	0.99	30.33	46.94	18.05	1.38
	7	70.21	0.66	90.60	3.65	18.75	1.36
	10	70.00	0.70	87.04	1.16	20.43	0.41
	15	133.75	0.31	115.16	0.28	52.28	0.25
GS vs. AE (r)		–0.9688		–0.4527		–0.7541	
A. anomala ARM 314	0	1.88	0.82	1.88	0.82	1.88	0.82
	4	5.95	0.90	5.88	0.10	0.42	3.60
	7	41.30	0.16	32.71	0.22	5.95	0.30
	10	29.50	0.17	37.57	0.25	18.35	0.13
	15	34.99	0.27	82.08	0.19	38.52	0.08
GS vs. AE (r)		–0.9559		–0.4411		–0.5686	
A. oryzae ARM 570	0	13.24	1.47	13.24	1.47	13.24	1.47
	4	42.73	1.86	30.64	48.02	15.31	0.94
	7	57.69	2.75	45.85	4.85	18.28	0.65
	10	47.06	0.70	45.36	1.30	42.24	0.17
	15	88.67	0.20	118.80	0.07	21.73	0.09
GS vs. AE (r)		–0.3675		–0.3077		–0.7027	

Table 2 (cont.)

Organisms	Time in days	N-sources					
		Control N≡N		KNO_3		$(NH_4)_2SO_4$	
		GS	AE	GS	AE	GS	AE
A. variabilis ARM 310	0	25.00	0.44	25.00	0.44	25.00	0.44
	4	2.18	1.48	2.60	0.71	8.17	0.75
	7	5.84	2.84	3.10	2.78	1.93	0.85
	10	4.97	1.92	3.06	2.21	2.62	1.07
	15	4.88	0.59	2.92	1.23	0.84	0.27
GS vs. AE (r)		–0.5199		–0.5660		–0.3304	
A. fertilissima ARM 742	0	54.12	1.24	54.12	1.24	54.12	1.24
	4	33.00	1.81	20.26	0.90	1.58	0.58
	7	5.94	0.44	5.64	1.07	0.76	0.36
	10	10.79	0.33	12.09	0.37	1.03	0.18
	15	12.66	0.30	12.90	0.21	1.61	0.05
GS vs. AE (r)		0.7545		0.3895		0.9647	

r – Correlation coefficient

Quantitatively, ARM 310 produced maximum protein whereas ARM 314 and ARM 742 had minimum quantity of protein (mg ml^{-1}) irrespective of the N-source provided in the growth medium.

Heterocyst frequency and nitrogenase activity

In general, heterocyst frequency varied from 3.17–6.50 per cent in the strains when grown on elemental nitrogen. Heterocyst differentiation was markedly affected by exogenously added combined nitrogen. Irrespective of the particular strain, strong inhibition in heterocyst frequency was observed in NH_4^+-N and by the 10th day all the filaments were devoid of heterocysts (Table 2). On the other hand, NO_3-N supplemented medium caused a gradual decrease in heterocyst frequency. In ARM 314, filaments were completely devoid of heterocysts by 7th day in NH_4^+-N but not in KNO_3-N even up to the 15th day. The other strains also showed a similar trend in heterocyst frequency, except for ARM 310 which showed complete absence of heterocysts by the 15th day even in KNO_3-N medium.

The strains grown on molecular N_2 showed a time dependent increase in nitrogenase activity up to the 10th day. Among all the five strains the highest acetylene reduction activity (ARA) of 3.77 and 1.01 μ mole C_2H_4 mg Chl^{-1} h^{-1} on the 10th and 15th day, respectively, was observed in *A. oryzae* ARM 570. In general ARA was markedly affected by the addition of different combined sources of nitrogen. Ammonium-N exerted a strong inhibition of nitrogenase activity (ARA) in all the five strains and by the 10th day there was only negligible activity. The nitrogenase activity also declined in presence of NO_3-N, but as low as 3 per cent and as high as 58 per cent ARA could be detected in ARM 310 and ARM 570 on the 15th day of growth (Table 2). A strong positive correlation (r) between the heterocyst frequency and nitrogenase activity could be observed in NH_4^+-N supplemented grown species of cyanobacteria. Similar but low positive correlation was seen in KNO_3-N followed with N_2 grown cultures.

GS activity and ammonia excretion

In molecular nitrogen grown cultures, GS activity increased with the increase in duration of incubation up to 15 days in both strains of *A. oryzae* (ARM 313 and ARM 570), whereas GS activity in *A. anomala* ARM 314 increased up to 7 days thereafter it declined marginally. In *A. variabilis* (ARM 310) and *A. fertilissima* (ARM 742) GS activity remained very low throughout the period of incubation (Table 3). Similar increasing trend in GS was shown in KNO_3-N and NH_4^+-N supplemented medium grown cultures of *A. oryzae* (ARM 313 and ARM 570) and *A. anomala* ARM 314, although the absolute quantity differed significantly. Among the five strains, *A. oryzae* ARM 313 showed the highest GS activity irrespective of the N-source in the medium (Table 3).

Ammonia excretion was mainly confined to the first 7 days of incubation in all except the ARM 310 strain. The excretion was variously influenced by different nitrogen sources e.g. in N_2-N maximum ammonia was excreted by *A. oryzae* ARM 570 (1.47–2.75 µ moles NH_4^+ mg^{-1} Chl) and *A. variabilis* ARM 310 (0.44–2.84 µ moles NH_4^+ mg^{-1} Chl) up to 7 days of incubation. In medium with N_2-N and NH_4^+-N a uniformly low excretion of ammonia was observed. Similar low excretion of ammonia was seen with ARM 742 and ARM 310 in NO_3-N grown cultures (Table 3). Hence it is clear from the results that there was significant amount of ammonia excretion in nitrate grown *A. oryzae* during the first four days of incubation. Significantly, except *A. fertilissima* ARM 742, a negative correlation (r) between glutamine synthetase and ammonia excretion was observed.

Table 4
Effect of different nitrogen sources on nitrate reductase activity (n mol mg^{-1} protein)

Anabaena sp. & ARM No.	Time in days	Nitrogen sources		
		N≡N	KNO_3	$(NH_4)_2SO_4$
A. oryzae 313	0	72.15	72.15	72.15
	4	31.65	191.77	20.39
	7	14.45	163.33	3.59
	10	5.16	137.11	1.80
	15	0	112.21	0
A. anomala 314	0	14.29	14.29	14.29
	4	4.84	49.81	13.73
	7	0	80.00	1.70
	10	0	78.14	0
	15	0	80.76	0
A. oryzae 570	0	26.83	26.83	26.83
	7	11.00	21.81	0
	7	0	76.84	0
	10	0	113.64	0
	15	0	49.63	0
A. fertilissima 742	0	44.91	44.91	44.91
	4	2.72	404.67	0
	7	0	177.94	0
	10	0	129.77	0
	15	0	194.16	0
A. variabilis 310	0	46.72	46.72	46.72
	4	0	277.51	5.78
	7	0	381.31	0
	10	0	593.31	0
	15	0	435.30	0

Nitrate reductase (NR) activity

In all the five strains NR activity was highest in nitrate supplemented medium. NR activity continuously increased with the increased duration of incubation up to 15 days in *A. fertilissima* ARM 742 and *A. anomala* ARM 314 in the presence of NO_3-N. Similar increase in NR activity up to 7 days was observed in *A. oryzae* ARM 313 and *A. variabilis* ARM 310 and on further incubation it declined or remained constant (Table 4). But in N_2-N and NH_3-N grown cultures, NR activity declined immediately and became almost zero within 4 to 7 days of incubation in all strains of *Anabaena* except *A. oryzae* ARM 313 wherein it took 10 days.

DISCUSSION

In general, nitrogen constitutes 1–2% of the total dry weight of plants and in unfertilized soils often limits crop production. The energy crisis in the early 1970s and the concomitant escalating cost of chemical N fertilizers led to the search for N-alternatives/supplements. In this context, the role of cyanobacteria which are self perpetuating organisms using solar energy and are predominant in rice paddies have been studied by many workers. The most abundant and well-studied group among cyanobacteria are the heterocystous *Anabaena* and *Nostoc*. This investigation therefore throws light on specific strains of *Anabaena* and their response to different nitrogen levels.

Cyanobacteria mainly use inorganic nitrogen compounds (NO_3^--N, N_2-N and NH_4^+-N) to fulfill their N-requirement but some strains assimilate other sources of nitrogen such as urea and amino acids. NO_3^--N has been considered more favourable for cyanobacterial growth [2, 6, 15]. In this investigation NO_3^--N also supported better growth of three out of five strains of *Anabaena* (ARM 313, ARM 570 and ARM 742) in terms of chlorophyll synthesis (Table 1). Singh and Srivastava [29] reported that NH_4^+-N supports poorer growth and may cause cell lysis accompanied by sudden drop in pH of the medium. However, in the present investigation the medium was supplemented with MOPS buffer, therefore, neither the pH was reduced nor the cells were lysed; rather, one of the strain (*A. variabilis* ARM 310) showed better growth in NH_3-N medium in terms of both chlorophyll and protein synthesis. *A. anomala* ARM 314 was the only strain that fared well when grown on N_2-N. Growth as protein content also showed a similar trend as chlorophyll in ARM 313 and in other strains with minor variations. Based on the logistics of cell economy, ammonium should be the most preferred N-source, since the assimilation of nitrogenous substrates shows an increase in energy requirement in the order: NH_4^+-N, NO_3^--N and N_2-N indicating the existence of regulatory interactions between the assimilatory processes.

Heterocysts (nitrogen fixing structures) make up about 5–7% of the total cells of *A. cylindrica* under nitrogen fixing conditions. This percentage can be increased by growth in the absence of a N source [7] or by adding inhibitors of glutamine syn-

thetase [16, 33]. With increasing heterocyst frequency above 7–9%, there is usually no corresponding increase in total nitrogenase activity per filament. Both heterocyst differentiation and nitrogenase synthesis are affected more strongly by NH_4^+-N rather than NO_3^--N [25, 36]. The NO_3^--N causes transitory and variable inhibition of nitrogenase activity and heterocyst differentiation [11, 24]. Similar responses with NO_3^--N have been observed in the *Anabaena* strains studied during the present investigation. In NO_3^--N supplemented medium, neither heterocyst frequency nor nitrogenase activity was completely inhibited except in ARM 310 by the 15th day of incubation (Table 3). However, in the NH_4^+-N medium all the five strains completely lacked heterocysts and had no nitrogenase activity. Bottomley and co-workers [4] indicated that 5 mM NO_3^--N repressed heterocyst differentiation but not the nitrogenase activity in already differentiated heterocysts. This may be the reason for reduced nitrogenase activity in the strains of *Anabaena* analyzed by us. Our study shows that N_2-fixation by cyanobacteria may not be inhibited in the presence of NO_3^--fertilizers commonly used in rice cultivation.

Stewart [36] reported that the GS is the primary ammonia assimilating enzyme and it occurs both in heterocysts and vegetative cells. The NH_4^+ produced in heterocysts is assimilated by glutamine synthetase (E.C. 6.3.1.2) which has a low K_m for NH_4^+ (1 mM), and can scavenge NH_4^+ from around nitrogenase [8]. Tracer studies [34, 37, 38] showed that glutamine is transferred to the vegetative cells, presumably via the micro-plasmadesmata, that traverse the plasmalemma between vegetative cells and the adjoining heterocyst pores. In vegetative cells, glutamate synthase (E.C. 1.4.7.1) which appears to be absent in heterocysts, converts glutamine to glutamate with some of the glutamate being transferred back to heterocyst to act as substrate for glutamine synthetase [37] and some being utilized in the vegetative cells for general amino acid synthesis. GS seems to be regulated by the nature of N-sources. The maximum GS activity was observed in cultures grown on N_2-N and NO_3^--N while its activity was markedly low in cultures grown on NH_4^+-N [31, 34]. During the present investigation maximum GS activity was observed in N_2-N or NO_3^--N whereas minimum GS activity was in NH_4^+-N (Table 3).

The selection of strains possessing non-repressible nitrogenase, rapid growth and liberation of substantial amount of ammonia is the main strategy for maximizing N gain through the use of cyanobacteria [9, 23]. *Anabaena* sp. grown on N_2-N excrete NH_4^+ in fairly large quantities in the first 7–10 days and thereafter the excretion is reduced suggesting a greater demand by the organism itself. This excretion of NH_4^+ was further enhanced in presence of NO_3^--N. In $(NH_4)_2SO_4$ medium a marginal increase in NH_4^+-N excretion was seen in *A. oryzae* ARM 313, *A. anomala* ARM 314 and *A. variabilis* ARM 310 (Table 3).

The high levels of excreted ammonia in NO_3-N grown cultures may be explained as the net result of initial enhancement of NR activity and continued nitrogenase activity in the presence of nitrate. The enhanced pool of nitrogen leads to excretion of ammonia, which is in excess of biosynthetic requirement. The process of NH_4^+-excretion does not seem to be growth linked as the maximum excretion by *A. oryzae* ARM 313 and ARM 570 in NO_3^--N medium occurred on the 4th day. On the other

hand *A. fertilissima* ARM 742 showed decreased NH_4^+-excretion with increase in growth period. Statistical analysis revealed that except for *A. variabilis* ARM 310, all the strains showed high nitrogenase activity and higher levels of NH_4^+-excretion, especially the two strains of *A. oryzae* ARM 313 and ARM 570. These strains therefore seem to be potentially useful for use as biofertilizers as their GS activities were also low in NH_4^+-N.

In the present investigation, the NR activity was higher in NO_3-N medium even when the heterocyst frequency was lower as compared to control. But in NH_4^+-N medium the NR activity was greatly reduced by the 4th day; this may be due to the effect of NH_4^+ on the high affinity transport system for NO_3^- which is known to be the primary target leading to cessation of NR activity [13].

It is well known that ammonia excretion is intricately related to GS activity, with low levels of GS enzyme being essential for maximum excretion of ammonia. A significantly high negative correlation was observed between these two parameters for all the *Anabaena* strains except *A. fertilissima* ARM 742. It would be interesting to study the GS enzyme of this strain because many mutants which are GS-deficient and ammonia-leaky in nature show poor growth attributes. Therefore, ARM 742 needs further investigation of its GS activity so that it may be exploited beneficially as an inoculant. Statistical analysis further corroborates earlier reports on the high positive correlation between heterocyst frequency and nitrogenase activity i.e. the appearance of nitrogenase activity goes with hand to hand with the development of heterocysts [5]. The relationship between ammonia and nitrogenase activity is rather complicated and the negative effect of ammonia is generally considered indirect [26]. In this context, C/N ratio seems to control the expression of enzymes involved in the utilization of different N-sources, as in enterobacteriaceae [18]. In this study, the correlation between GS and nitrogenase activity seems to be rather complex, showing a high positive correlation in KNO_3 medium for ARM 313, ARM 314 and ARM 570 but negative "r" values in case of ARM 310 and ARM 742, indicating different mechanisms in different strains. This investigation indicates the potential of *Anabaena variabilis* ARM 310, a strain capable of leaking out maximum amount of fixed-N. Our work also points to the necessity for an in depth analysis into the relationship between GS enzyme activity and nitrogenase activity for better utilization of cyanobacterial strains in biofertilizer production.

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