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Nitrogenase Derepression and Associated Metabolism in a Microaerophilic Cyanobacterium, *Plectonema boryanum*

PANDEY, KAPIL DEO*, SARKAR SUKLA, SHAHEEN NAZ, CHATURVEDI SMITA, AND KASHYAP AJAI KUMAR

Centre of Advance Study in Botany, Banaras Hindu University, Varanasi 221 005, India

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Abstract Nitrate grown cells of cyanobacterium *Plectonema boryanum*, transferred to nitrogen stress, evolved nitrogenase catalyzed H_2 under microaerophilic condition. Nitrogen (N_2) in gas phase, low light intensity, and reducing substances in incubation phase stimulated N_2 fixation (H_2 evolution). Cyanobacterium grew slowly under microaerobic condition with a low intracellular ammonia pool. Nitrogen sources (NO_3^-, NH_4^+, and CH_3NH_3) inhibited nitrogenase and glutamine synthetase (GS) transferase activity, and methylamine behaved like an ammonical nitrogen source. Depletion of molybdenum (Mo) and addition of tungsten (W) in the incubation medium inhibited H_2 evolution. Cyanobacterium was able to take up nitrate and expressed nitrate reductase (NR) activity under microaerophilic condition at an extremely slow rate.

Key words: *Plectonema boryanum*, nitrogenase derepression, H_2 evolution, physiological factors, aspect of regulation

Among the cyanobacteria, the oxygen evolving photoautotrophs, diazotrophy is a wide-spread phenomenon. As a result of intensive physiological, biochemical, and genetical investigations on heterocystous cyanobacteria, it is generally agreed that heterocysts contain nitrogenase and, therefore, are the site of nitrogen (N_2) fixation [10]. *Symploca* and *Trichodesmium* fix nitrogen aerobically in a light/dark cycle, and *Leptolyngbya* does so under anaerobic condition, even though they do not form heterocyst. The *het R* gene, a key regulatory gene in heterocyst differentiation, has been sequenced in all three cyanobacteria [12]. It has also been demonstrated that, besides dinitrogen reduction, the nitrogenase enzyme catalyzes hydrogen (H_2) evolution (proton reduction) [3], and due to this reason, heterocystous cyanobacteria were considered as a potential tool in hydrogen production biotechnology. However, the uptake hydrogenase located in heterocysts recycles evolved H_2 [15], thus saving energy on one hand and lowering H_2 production on the other. The level of bidirectional enzyme transcript did not change significantly during the induction of H_2 uptake activity in *Nostoc muscorum* [2].

While N_2 fixation appears to be prevalent in heterocystous cyanobacteria, some of the non-heterocystous forms have been demonstrated to fix N_2 aerobically, microaerobically, and anaerobically [7]. In *Gloeothece* sp., nitrogenase activity appeared in the dark phase when the cells were grown under an alternating light/dark cycle of 12 h. The synthesis of both Fe protein and MoFe protein commences immediately prior to the appearance of activity. Degradation of nitrogenase proteins starts as early as 4 h in the dark period and continues in the light phase. Consequently, both nitrogenase proteins are absent during most of the light phases. The two proteins of molecular weight of 47,000 and 29,000 are specially synthesized during this period, and it is possible that they have a role in nitrogenase degradation. [26]. In all N_2-fixing non-heterocystous cyanobacteria, whether or not they can fix N_2 under oxic conditions, the *nif* structural genes are contiguous and arranged in the order *nif HDK*. [4].

It appears that a culture of a non-heterocystous cyanobacterium contains 10–20 times more nitrogenase than that in equivalent culture of heterocystous cyanobacterium [4]. Organisms such as *Oscillatoria limosa* and *Plectonema boryanum*, which grow aerobically in the presence of a combined inorganic nitrogen source, develop nitrogenase in all of the cells of the filament if shifted to microaerobic condition [34, 25, 4]. It is therefore, anticipated that non-heterocystous cyanobacteria should evolve more hydrogen than the heterocystous species. However, in comparison to the latter forms, H_2 evolution by non-heterocystous cyanobacteria represents a two-step process, i.e. initially they must be cultivated in the presence of a combined inorganic nitrogen source (Step I) followed by incubation in the absence of a

*Corresponding author
Phone: 91-0542-30-7147; Fax: 91-0542-317074;
E-mail: kashyap@banaras.ernet.in

combined nitrogen source in an inert atmosphere (Step II). Factors such as light intensity, nutritional conditions, and composition of the gas phase that may affect hydrogen production during Step II, and appear to be important in the development of H_2 production, were investigated in *Plectonema boryanum*. Results on nitrogenase derepression and H_2 evolution in this non-heterocystous cyanobacterium have been presented.

MATERIALS AND METHODS

Organism and Growth

Plectonema boryanum (UTEX 594; PCC 7311) was cultivated in modified Chu No.-10 medium [28] in a culture room maintained at 25±1°C and illuminated with daylight fluorescent tubes (photon flux 50 μE m^{-2} s^{-1}) for 14 h a day.

Growth was estimated at regular interval of one day by determining protein content per ml. Generation time (G) was determined following the formulae of Myers and Kratz [18].

Nitrogenase Derepression

Exponential phase cells of cyanobacterium concentrated through centrifugation was resuspended in a nitrogen-free medium in a bottle or in venoject vacuum blood collection tubes (Terumo Medical Corporation, U.S.A.), sealed with air tight rubber stopper/septum caps (cell density 150 μg protein ml^{-1}). According to experimental design, these were flushed with Argon (Ar): CO_2 (96:4, v/v), Ar:N_2:CO_2 (72:24:4, v/v) gas mixtures for 15 min at 0, 6, and 12 h of incubation or every 12 h intervals. The cultures were incubated in continuous illumination (25 μE m^{-2} s^{-1}). These bottles or tubes were used for H_2 measurement and acetylene reduction assay without transferring the cultures to other vessels in order to avoid air contamination.

Enzyme Assay

Nitrogenase activity. Nitrogenase activity (C_2H_2 reduction) was measured by the methods of Scholhorn and Burris [30] in a gas chromatograph (Tracor 540, U.S.A.). The amount of C_2H_4 was detected using 99.5% pure ethylene (Scott Speciality Gases, U.S.A.).

H_2 measurement. H_2 evolution was analyzed in a gas chromatograph on a Thermal Conductivity Detector (TDC) mode. Argon gas served as the carrier and the analysis was performed at 110°C.

Glutamine synthetase (GS). GS-transferase activity was measured colorimetrically following the method of Shapiro and Stadtman [31]. The enzyme activity was quantified from the amount of γ-glutamyl hydroxymate formed by the substrate.

Nitrate reductase (NR). Nitrate reductase (NR) activity in the cells was determined following the method of Manzano *et al.* [17]. Nitrate formed during the reaction was determined by an azocoupling reaction using the method of Snell and Snell [33].

Analytical Methods

Protein. Protein was determined following the method of Lowry *et al.* [16] taking lysozyme (Sigma, U.S.A.) as the standard.

Ammonium. Intracellular ammonium pool was determined by a modified method of Kleiner [14] employing the phenate hypochloride method [13].

Nitrate. Nitrate was determined by the brucine-sulphuric acid method [20].

Amino acid pool. Amino acid pool of the cyanobacterial cells was estimated according to the method of Sutherland and Wilkinson [36] employing ninhydrin reagent. L-glutamine was used as the standard.

Dry weight. Dry weight measurement was based on the conventional overdrying of cyanobacterial suspension at 80°C in an incubator till constant weight.

Photosynthetic Pigments

Chlorophyll (Chl) *a* was extracted in 80% acetone at room temperature overnight. The remaining pellet, after evaporation of the acetone, was used for phycocyanin (PC) extraction in water employing the freezing and thawing method. The pigments were quantified (μg mg^{-1} protein) using specific absorption coefficients (α) 82.04 and 7.5 of Allen [1] and Brody and Brody [5] for Chl *a* and phycocyanin, respectively.

RESULTS

Derepression of Nitrogenase Activity (H_2 Evolution)

Nitrogenase activity (C_2H_2 reduction) in *P. boryanum* was depressed after 24 h and reached to its maximum following 48–60 h of incubation in a micro-oxic growth condition. In a parallel set of experiment where C_2H_2 was not added in the gas atmosphere, cyanobacterium evolved H_2 which followed a pattern similar to nitrogenase activity. Since C_2H_2 reduction and H_2 evolution followed a parallel trend, it was concluded that H_2 evolution in the cyanobacterium was nitrogenase mediated (Fig. 1).

Effect of Gas Composition

Nitrogenase derepression in *P. boryanum* required microaerobic incubation as no nitrogenase activity was observed in the air/CO_2 gas phase (medium devoid of NO_3^- nitrogen). Microaerobic conditions created by using either Ar/CO_2 or N_2/CO_2 (96/4, v/v) resulted in 27% higher nitrogenase activity in the latter gas atmosphere than the former. In another treatment, both the inert gases were mixed in a 3:1 ratio and the nitrogenase activity was nearly 2.3 and 1.7 times higher in the Ar/N_2/CO_2 gas phase than the Ar/CO_2

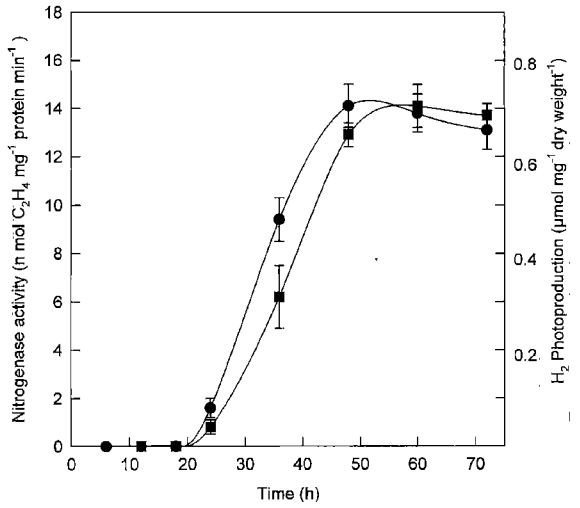

Fig. 1. Derepression of nitrogenase activity (■) in *Plectonema boryanum* when nitrate aerobic grown cells were transferred to a combined inorganic nitrogen starved medium under microaerobic ($Ar/N_2/CO_2$, 72/24/4; v/v) condition.
H_2 photoproduction (●) was measured in a parallel set of experiments under a similar set of conditions as above. Values are the mean of four independent experiments±S.D.

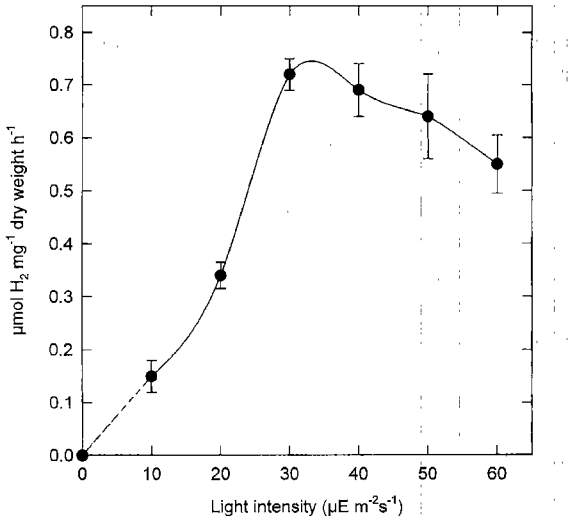

Fig. 2. Effect of different light intensities on H_2 evolution by *Plectonema boryanum*. Experimental conditions were similar to those described in Fig. 1.

Table 1. Nitrogenase activity and photoprodution of H_2 by *Plectonema boryanum* under different gas phase incubations (measurements at 48 h).

Gas phase (v/v)	Nitrogenase activity[1]	Hydrogen evolution[2]
Air/CO_2 (96/4)	ND	ND
N_2/CO_2 (96/4)	8.1±0.9	0.44±0.03
Argon (Ar)/CO_2 (96/4)	5.9±0.4	0.30±0.03
$Ar/N_2/CO_2$ (72/24/4)	13.8±1.1	0.72±0.05

1=nmol C_2H_4 mg^{-1} protein min^{-1}.
2=µmol H_2 mg^{-1} dry wt h^{-1}.
ND=not detectable.

and N_2/CO_2 gas phases, respectively. H_2 evolution under the $Ar/N_2/CO_2$ and N_2/CO_2 gas phases was 2.5 and 1.5 times higher than the Ar/CO_2 gas phase (Table 1).

Effect of light intensity. *P. boryanum* exhibited maximum growth in the NO_3^- supplemented medium at a fluence rate of 50 µE m^{-2} s^{-1}. The response of H_2 evolution in relation to light intensity has been represented in Fig. 2. H_2 evolution at 10 µE m^{-2} s^{-1} was 0.15 µmol mg^{-1} dry wt h^{-1} and the rate increased upto 30 µE m^{-2} s^{-1} (0.72 µmol H_2 mg^{-1} dry wt h^{-1}). Further increase in light intensity inhibited the H_2 evolution (Fig. 2). The results indicated that the low light intensity supported N_2-fixation (measured in terms of H_2 evolution) in the cyanobacterium.

Effect of reducing substances. In order to enhance the low nitrogenase activity (2.2 nmol C_2H_4 mg^{-1} protein min^{-1} at 60 h of incubation) of *P. boryanum*, reducing substances sodium sulfide or dithionite (2 mM each) were supplemented at the beginning of the microaerobic incubation. The nitrogenase activity increased to 6.5 and 9 fold in the presence of sulfide and dithionite, respectively, over the control (no reducing substances). Dithionite, which stimulated maximum N_2 fixation, enhanced H_2 production by 5 fold, whereas sulfide supported the highest H_2 evolution (6 fold) compared to the control (0.12 µmol mg^{-1} dry wt h^{-1}) (Table 2).

The photosynthetic pigment ratio (phycocyanin (PC)/ Chlorophyll *a* (Chl *a*)) in the cyanobacterium grown under aerobic condition (NO_3^- medium, control) was 9.4. The value declined sharply to 1.3 under microaerobic condition (-N), indicating that PC degradation was initiated. Addition of

Table 2. Effect of reducing substances on the nitrogenase activity, H_2 evolution, and intracellular pool of photosynthetic pigments, ammonium, and amino acid of *Plectonema boryanum**.

Conditions	Nitrogenase activity[1]	Hydrogen evolution[2]	PC/Chl a ratio	Ammonium pool[3]	Amino acid pool[4]
Control (aerobic, nitrate)	ND	ND	9.4±0.0	0.24±0.02	57.6±4.5
$Ar/N_2/CO_2$ (74/24/4)	1.7±0.04	0.12±0.01	1.3±0.1	0.09±0.10	10.0±1.8
$Ar/N_2/CO_2$+sulfide (2 mM)	12.4±0.90	0.72±0.04	8.1±0.5	0.13±0.02	28.5±1.4
$Ar/N_2/CO_2$+dithionite	17.6±0.90	0.63±0.05	7.3±0.3	0.12±0.01	31.2±2.6

*measured at 48 h.
1=nmol C_2H_4 mg^{-1} protein min^{-1}; 2=µmol H_2 mg^{-1} dry wt h^{-1}.
3=mM; 4=µg mg^{-1} dry wt.

sulfide or dithionite could restore PC degradation to a level of 86% and 78%, respectively, in comparison to the control (Table 2).

P. boryanum grown aerobically exhibited a 0.24 mM NH_4^+ pool and 57.6 μg mg^{-1} dry wt water soluble amino acid pool measured at 96 h of incubation. The intracellular NH_4^+ pool declined sharply under the N_2-fixing condition (38% of the nitrate grown cells). Addition of sulfide and dithionite in microaerophilic cultures slightly restored the amino acid pool although no significant difference was observed in the intracellular NH_4^+ pool (Table 2).

Growth of *P. boryanum* under Microaerophilic Condition

Cyanobacterium grown aerobically in nitrate medium exhibited a doubling time of 22 h. The increase in growth under the diazotrophic condition was meagre, and in comparison to sulfide growth, was better in the dithionite supplemented medium. At the inoculum protein of 110 μg ml^{-1}, the increase in protein content was nearly 1.5 and 1.8 times in the sulfide and dithionite supplemented medium, respectively, whereas it was 7.5 times higher in the nitrate medium. The results indicated that N_2-fixing growth was insignificant compared to nitrate-supported growth (Fig. 3).

Effect of Nitrogen Sources on Nitrogenase Activity and GS-Transferase Activity

To observe the effect of nitrogen sources on the nitrogenase activity of *Plectonema boryanum*, diazotrophic cultures (60 h) after centrifugation and washing were incubated in nitrate (5 mM KNO_3), ammonia (2.5 mM NH_4Cl), and methylamine (3.7 mM CH_3NH_3) supplemented media under microaerobic condition in separate treatments. All the nitrogen sources inhibited nitrogenase activity (Table 3). Inhibition in activity by nitrate was low (10% of the zero h activity), whereas ammonium repressed the nitrogenase activity maximally (58%). Methylamine behaved like ammonical nitrogen in repressing C_2H_2 reduction activity, although the inhibition was comparatively less.

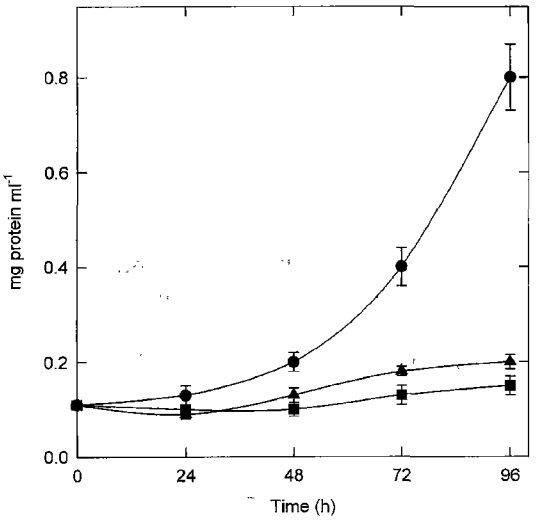

Fig. 3. Growth of *Plectonema boryanum* under aerobic (●) (nitrate, $Ca(NO_3)_2 \cdot 4H_2O$, 1 mM) and microaerobic (-N) conditions. Gas sparging in microaerophilic condition was as in Fig. 1. Reducing agent sodium sulfide (■) or dithionite (▲) was added at the beginning of the incubation.

Table 3. Effect of nitrogen sources on the GS-transferase and nitrogenase (C_2H_2 reduction) activities of *Plectonema boryanum.*

Conditions	GS-transferase activity[1]	C_2H_2 reduction[2]
Aerobic nitrate medium	250	0.0
Microaerobic ($Ar/N_2/CO_2$, 72:24:4,v/v)	298	15.0
$Ar/N_2/CO_2$+KNO_3 (10 mM)	287	13.0
$Ar/N_2/CO_2$+NH_4Cl (2.5 mM)	207	6.3
$Ar/N_2/CO_2$+Methylamine (4 mM)	232	9.0

1=nmol γ-glutamyl hydroxymate mg^{-1} protein min^{-1}.
2=nmol C_2H_2 mg^{-1} protein min^{-1}.
Activities were determined after 4 h of addition.

Nitrogen sources also inhibited GS-transferase activity. Ammonium and methylamine repressed the GS activity in a similar manner as was observed for C_2H_2 reduction. The inhibition was more pronounced by NH_4Cl (30%) compared to methylamine (22%). The observation that nitrate did not inhibit nitrogenase activity could be due to the fact that nitrate should be reduced to NH_4^+ before exerting its effect on nitrogenase activity. The results therefore indicated the possibility of a low level of NR induction in N_2-fixing cells (Table 3).

Effect of Mo, W, and Pd on Nitrogenase Activity and H_2 Evolution

In order to confirm that H_2 evolution in *P. boryanum* was nitrogenase mediated, sodium tungstate, an inhibitor of nitrogenase, was added with or without Mo to observe its effect on H_2 evolution and nitrogenase activity. The cultures grown in the absence of Mo exhibited only 11% nitrogenase activity compared to the control (+Mo). Addition of tungsten (5 μM) to such cultures still lowered the nitrogenase activity which was only 6% of the control level. Inhibition by tungsten was less pronounced when it was added in Mo containing cultures (17% activity of the control). H_2 evolution followed a similar trend to those of nitrogenase, which indicated that H_2 evolution in this cyanobacterium was nitrogenase mediated.

Palladium (Pd) oxide is used as an H_2 absorbent and its application in mini H_2 fermentors has been proposed. Addition of Pd (5 μM) to the cultures grown in the absence of Mo could restore nitrogenase activity compared to the Mo-less control, however, when supplemented with Mo cultures, it exhibited only 58% activity compared to the

Table 4. Effect of molybdenum (Mo), tungsten (W), and palladium (Pd) on nitrogenase activity and H_2 evolution by *Plectonema boryanum**.

Conditions	Nitrogenase activity[1]	Hydrogen evolution[2]
-Mo	1.5±0.2	0.11±0.02
-Mo+W	0.8±0.1	0.06±0.01
-Mo+Pd	3.3±0.4	0.02±0.02
+Mo	13.8±1.1	0.72±0.04
+Mo+W	2.3±0.03	0.15±0.01
+Mo+Pd	8.0±0.05	0.47±0.05

*measured at 48 h.
1=nmol C_2H_4 mg^{-1} protein min^{-1}.
2=μmol H_2 mg^{-1} dry wt h^{-1}.
Mo (0. 5 μM), W and Pd (5 μM).

control (+Mo). H_2 evolution followed a similar trend and the values were 65 and 82% in the absence and presence of Mo and Pd added conditions compared to control, respectively (Table 4).

Induction of Nitrate Reductase Activity under Microaerophilic Condition

Nitrate uptake and nitrate reductase (NR) were investigated in diazotrophic and nitrogen starved cells of *P. boryanum*. The results presented in Fig. 4 indicated that nitrate uptake by N_2-fixing cells under microaerobic condition was nearly 14% of the N-starved cells (aerobic) at 20 h of nitrate (100 μM) addition in the medium. NR activity in the N-starved cells (aerobic) appeared between 4–8 h following incubation in 10 mM KNO_3 and steadily increased up to

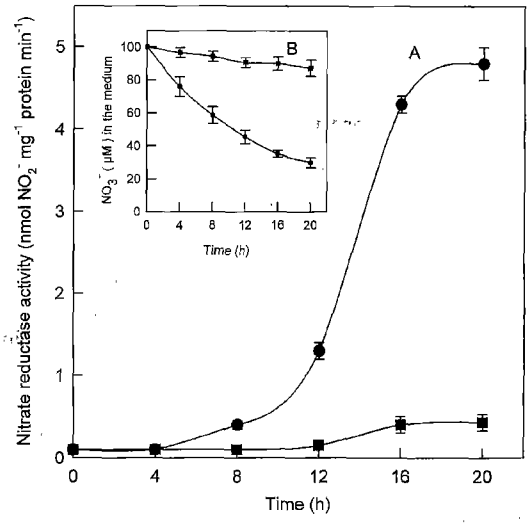

Fig. 4. Induction of nitrate reductase (A) and depletion of nitrate from the medium (B) on transfer of N_2-fixing *Plectonema* cells to nitrate medium (■).
Nitrogenase was derepressed as in Fig. 1. Nitrate grown aerobic cultures were nitrogen starved for 24 h and uptake and reductase activities were monitored under aerobic conditions (●). Values are the mean of 3–4 independent experiments and the bar on each point represents standard deviation.

16 h reaching to a value of 4.3 nmol NO_2^- mg^{-1} protein min^{-1}. Although induction of nitrate reductase activity was noticed at 16 h of incubation in diazotrophic cells, the activity was only 10% of the aerobic-grown cells. The slow nitrate uptake by N_2-fixing cells under microaerobic condition, and the consequently low rate of NR-activity, implies that nitrate may not be exploited for ensuring the growth of cyanobacterium under microaerobic conditions.

DISCUSSION

Even though aerobic N_2 fixation has been demonstrated in a few non-heterocystous cyanobacteria, most fix N_2 microaerobically where the O_2 concentration is less than 0.2 atm [7, 32]. The physiological changes, like nitrogen depletion and anerobiosis, required at the time of transformation of vegetative cells into 'heterocysts' [9], also exist with *P. boryanum* during the derepression of nitrogenase activity. The lag period required (18–24 h) seems to be related to nitrogen depletion of the cells. The observations are consistent with those of Stewart and Lex [35] and Rai *et al.* [25], and was further supported by the results on the intracellular ammonia pool which declined to nearly half in diazotrophic cells. The aerobic incubation of *P. boryanum* cells in N-free medium for 24 h, prior to transfer to a microaerophilic condition, resulted in the derepression of nitrogenase within 2–3 h [4]. Thus, nitrogen starvation of the cells seems to be the first and foremost requisite for nitrogenase derepression or photoproduction of H_2 by *P. boryanum*.

Nitrogen gas, used to create the anaerobic condition in the place of argon [15], inhibited H_2 evolution due to competition of N_2 reduction with proton [11]. Nitrogenase activity developed more quickly in the $N_2/Ar/CO_2$ gas phase than Ar/CO_2 or N_2/CO_2 gas phases [35]. The results that N_2 gas as a substrate is not necessarily required for nitrogenase derepression in *P. boryanum* is in accordance with others [35, 25]. The observation that N_2 in the gas phase stimulated H_2 evolution indicated that cyanobacterium evolved H_2 as a by-product of N_2 fixation ($Ar/N_2/CO_2$), and in the absence of N_2 gas (Ar/CO_2), it evolved H_2.

The possibility that uptake hydrogenase might be operative in *Plectonema* cells was overcome by inclusion of sulfide, known to inhibit H_2 recycling in *Nostoc muscorum* [38]. Nevertheless, uptake hydrogenase has yet to be demonstrated in any of the non-heterocystous cyanobacteria, with the possible exception of *Anacystis nidulans* which catalyzed both uptake of H_2 with $NAD(P)^+$ and H_2-evolution with NAD(P)H [29]. The cyanobacterium is incapable of anoxygenic photosynthesis [8]. It seems that *P. boryanum* may be able to oxidize hydrogen sulfide to sulfur, facilitating N_2-fixing growth [21]. The HS^- and H^+ ions liberated after dessociation of sulfide might have enhanced H_2 evolution

in *P. boryanum*. Dithionite-supported N_2 fixation was consistent with the observations of Nagatani and Haselkorn [19] who used dithionite as the O_2 scavenger in their studies.

The H_2 evolution in *P. boryanum* mediated by the nitrogenase enzyme which was supported by the following circumstantial evidences, loss of C_2H_2 reduction (H_2 evolution) following exposure of derepressed cultures to (i) NH_4^+, (ii) dark, and (iii) air. Our additional observation regarding the effect of Mo replacement on H_2 evolution further strengthens the above view. The results that *P. boryanum* possessed a low level of activity under Mo stress are consistent with the reports of Nagatani and Haselkorn [19].

Depending on the culture conditions, *P. boryanum* seems to be capable of photoautotrophic growth either with repeated phases of N_2 fixation or concomitantly with continuous N_2 fixation [25]. However, the results presented here did not show any cyclic phases of changes in protein content (growth or N_2 fixation), which might be attributed to the facts that (i) time intervals selected for measurement were too long to show such repeated cycles, and (ii) dithionite or sulfide used in the various experiments acted as O_2 scavenger and O_2 evolution inhibitor to some extent, respectively. *P. boryanum* could grow photoautotrophically using N_2 as a sole nitrogen source in an appropriate micro-oxic natural habitat [4].

The primary roles of light in the photosynthetic system are (i) to generate ATP through photophosphorylation and to generate reductant through the photosystem I-dependent process, and (ii) to generate reductant from water. The rate of H_2 evolution saturated at lower light intensities in *Oscillatoria* BG7, and there seemed to be a quantum threshold for H_2 evolution at low light as well as a minimum energy charge requirement for H_2 production [22]. In *P. boryanum*, nitrogenase synthesis can be derepressed at a low light intensity of 500 lx [25]. Low light intensity also supported H_2 evolution in other cyanobacteria [11].

Like heterocystous cyanobacteria, glutamine synthetase-glutamate synthase (GS/GOGAT) is the main route of primary ammonia assimilation in *P. boryanum*. An increased amount of GS in heterocyst is linked to nitrogenase synthesis and is necessary for N_2-derived ammonia assimilation [27]. Although an increase in GS-transferase activity with derepression of nitrogenase in *P. boryanum* is consistent with the above view, the corresponding increase was low compared to heterocystous forms. The results that NH_4^+ inhibited GS-transferase activity in *P. boryanum* are in accordance with the observations of Tuli and Thomas [37], who found that ammonical nitrogen grown cells of *Anabaena* L-31 possessed only half of its GS activity compared to N_2-fixing cells. The decrease in amino acid pool seems to be related to intracellular NH_4^+ produced either through N_2 fixation or NO_3^- reduction. The inhibition of nitrogenase activity by methylamine in *P. boryanum* has not been reported earlier. Methylamine could cause a repression only when it is assumed that it is transported and metabolized in the cells. Prakasham and Rai [23] considered *Nostoc* ANTH as an exceptional cyanobacterium where it is catabolized. The results in the present communication that methylamine repressed nitrogenase activity further indicated that it is catabolized by *P. boryanum* as a nitrogen source. The cyanobacterium could grow when methylamine was furnished as a nitrogen source under aerobic condition (data not presented).

Nitrate uptake and reductase (NR) activities were found to be inducible by substrate with a very low activity in nitrogenase derepressed cells, indicating that the cyanobacterium was able to minimize competition between nitrogenase and NR under diazotrophic conditions. The possibility that the low activity of NR was due to the low uptake of nitrate cannot be ruled out. However, it is noteworthy that N_2-fixing cells of cyanobacterium do have the capacity to develop nitrate uptake and NR, unlike the heterocyst where these systems are lost [24]. Cheng *et al.* [6] showed that the ability to assimilate nitrate was present in N_2-fixing cells of *Gloeothece* but not in ammonium grown cells. However, the presence of nitrate is not strictly required in order to induce its own assimilation system in *P. boryanum* [25].

The above studies indicated that regulation of nitrogenase activity in cyanobacterium *P. boryanum* could lead to H_2 photoproduction, but it needs further investigations, especially on the longevity and sustenance of cells during microaerobic conditions.

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