Isolation and Partial Characterization of Sodium-Azide Resistant Mutant Strain of Anabaena Cylindrica with Increase Growth and Heterocyst Differentiation

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Abstract

Sodium azide resistant mutant of diazotrophic cyanobacterium *Anabaena cylindrica* was isolated by N-methyl-N-nitro-N-nitrosoguanidine (NTG) mutagenesis and selected for sodium azide resistance. Sodium azide (NaN_3) is a general inhibitor of growth and respiration. The effect of NaN_3 (20 µg/ml) on growth, heterocyst differentiation, was studied by maintaining wild-type A. *cylindrica* as reference. The NaN_3 -tolerant mutant grew reasonably well both in presence and absence of NaN_3. It also produced more heterocysts. Exposure of wild-type A. *cylindrica* to NaN_3 (20µg/ml) for 7 days resulted in significant inhibition of growth and heterocyst differentiation. These activities were, however, significantly stimulated by NaN_3 in NaN_3-tolerant mutant.

Key Words - *Anabaena cylindrical*, Sodium-azide, Mutant strain, Growth, Heterocyst.

Introduction

Cyanobacteria are probably one of the most important groups of organisms. This is due to their global importance as they populate many environments. In Asia and the Far East, N_2-fixing cyanobacteria have been used as source of biofertilizer in rice-fields since early (De, 1939; Watanabe, 1956; Singh, 1961). One of the technological aspects receiving considerable attention, especially by using diazotrophic cyanobacteria, is the photobiological production of ammonia. However free-living diazotrophic cyanobacteria fix dinitrogen sufficient for their own needs and do not generally liberate significant amounts of ammonia into their external environments. The attempt are being made to induce ammonia production either by the addition of GS inhibitor or glutamate analogues such as L-methionine-DL-sulfoximine1 (MSX), 5-hydroxylysine, and phosphinothricin or by producing ammonia liberating mutant strains. By using MSX, continuous photoproduction of ammonia by diazotrophic cyanobacterium *Anabaena* have been reported (Chauhan *et al*., 1999a). In the absence of either a natural or ammonia liberating mutant strain, treatment of N_2 fixing cyanobacterial cells with GS inhibitors seem to be the preferred way for induced ammonia liberation. However, prolonged exposure to MSX has been reported to be toxic for cyanobacteria, causing nitrogen starvation and inhibition in growth and protein synthesis. By using chemical mutagens such as N-methyl-N-nitro-N-nitrosoguanidine (NTG), several cyanobacterial mutant strains resistant to MSX or defective in GS have been isolated and shown to liberate ammonia in the external medium. However, these mutant strains are slow growing and cannot withstand the environmental extremes like NaCl stress. NaCl resistant mutant strain of the cyanobacteria *Anabaena variabillis* by NTG mutagenesis possessing high NaCl tolerance had also been reported (Chauhan *et al.*, 1999). Azide is an alternate substrate for nitrogenase and is reduced to ammonia and elemental nitrogen by the enzyme (Schollhorn and Burris, 1967). Thus, it is well known for its use in

pesticide formulation. The first known and recorded use of sodium azide as a pesticide was in the 1920 (Richards, 2006). It is also a general inhibitor of growth and respiration (Hardy and Knight, 1967). Sodium azide is also used as pesticide in rice fields along with carbofuran, propanil, bifenex. The response of cyanobacteria to sodium azide stress will give insight into how and when azide can limit their growth in situ. An attempt has, therefore, been made to develop a diazotrophic cyanobacteria strain which can withstand azide concentration, can fix nitrogen at higher rate and can release most of the fixed nitrogen as ammonia.

The present investigation is an attempt to isolate and characterize a sodium azide resistant mutant strain of diazotrophic cyanobacterium *A cylindrica*. The isolated mutant may exhibit heterocyst differentiation and specific growth rate in presence of NaN_3 as compared to its wild-type.

Material and Methods

Maintenance of cyanobacterial culture

Wild type and mutant strain of *Anabaena cylindrica* were axenically grown in BG11 medium (Rippka *et al.*, 1979) without nitrogen source. Cultures were incubated in a culture room at 25±1°c and illuminated with day-light fluorescent tubes having photon fluence rate of 50 µmol m^2 s^{-1} at surface of the vessels. Unless otherwise stated, all the experiments were carried out with mid-log phase cultures. The medium was buffered to pH 7.5 with 10 mM Hepes/NaOH.

Mutagenesis & isolation of sodium azide resistant mutant strain

Exponentially grown cells of *A. cylindrica* were harvested by centrifugation and resuspended in 10mM HEPES/NaOH (pH 7.0).N-methyl-N-nitrosoguanidine (NTG, 250µg ml^{-1}) was added to the cultures (Chauhan *et al.*, 2001). The cultures were incubated at 30°c for 4 hrs in light with shaking. The cultures were washed three times with BG11 medium and resuspended in same medium. Mutagenized cells were grown photoautotrophically for several generations and transferred into liquid BG11 medium containing 5mM KNO3 with 20mg/100ml sodium azide. The strain thus isolated showed more tolerance against sodium azide and designated as sodium azide resistant mutant.

Heterocyst frequency

Heterocyst were counted microscopically in ten independent replicates and expressed as number of heterocysts per hundred vegetative cells.

Measurement of growth

Growth of *A. cylindrica* and its sodium azide resistant NaN^r_3 mutant strain was monitored by measuring the increase in chlorophyll a (chl a). It was expressed in terms of specific growth rate (µh-1). The specific growth rate constant (µ) corresponds to ln 2/td, where td is the doubling time.

Result and discussion

The objective of this study was to isolate and develop sodium azide(NaN_3) resistant (NaN^r_3) strain of diazotrophic cyanobacteria *A.cylindrica* by NTG mutagenesis and selection for NaN_3 resistance. NaN^r_3 mutant strain of *Anabaena cylindrica* developed in this study was compared with its wild type strain in presence as well as in absence of NaN_3 .The wild type *A. cylindrica* and its NaN^r_3 mutant counterpart exhibited similar growth patterns when cultured with N_2, NO^-_2, NO^-_3 , and glutamine in the absence of NaN_3 (Fig. 1). This clearly indicates that wild type *A. cylindrica* and its NaN^r_3 mutant counterpart grew reasonably well with all nitrogen sources in the absence of NaN_3. Growth of the wild type *A. cylindrica* (in terms of *chl* a) was completely arrested within 10 days at 20 µg ml-1 NaN_3 irrespective of the presence of

nitrogen sources (Fig. 2). In contrast, NaN^r_3 mutant strain grew reasonably well in the presence of NaN_3 with different nitrogen sources (N_2, NO^-_2, NO^-_3, NH^+_4 and glutamine). Further, NaN^r_3 mutant counterpart of *A. cylindrica* exhibited maximum and negligible growth in presence of NaN_3 in and containing media, respectively (Fig. 3). These results clearly indicate that growth of mutant strain of *A. cylindrica* was not inhibited by NaN_3 concentration and it can synthesize chlorophyll in the presence of N_2, NO^-_2, NO^-_3 and glutamine except NH^+_4. Similarly azide (20µg/ml) also completely inhibited the growth of algae in presence of different nitrogen sources (Singh and Singh, 1978). Table 1 represents the data on heterocyst differentiation in NaN^r_3 mutant strain and its parent, *A. cylindrica*. It is evident that mutant strain produced heterocysts in the presence of all nitrogen sources, maximum being in N_2 followed by glutamine, NO^-_2, NO^-_3, NH^+_4 and . In contrast, wild type *A. cylindrica* could not produce heterocyst in the presence of nitrogen sources except glutamine showing heterocyst differentiation of 2.2% only. Heterocyst differentiation in NaN^r_3 mutant strain of *A. cylindrica* was about two fold higher in the presence of N_2 and glutamine as compared to its wild type counterpart. NO^-_3 and NH^+_4 are known to adversely affect heterocyst differentiation in heterocystous cyanobacteria (Bothe, 1982; Del Campo *et al.*, 1988).

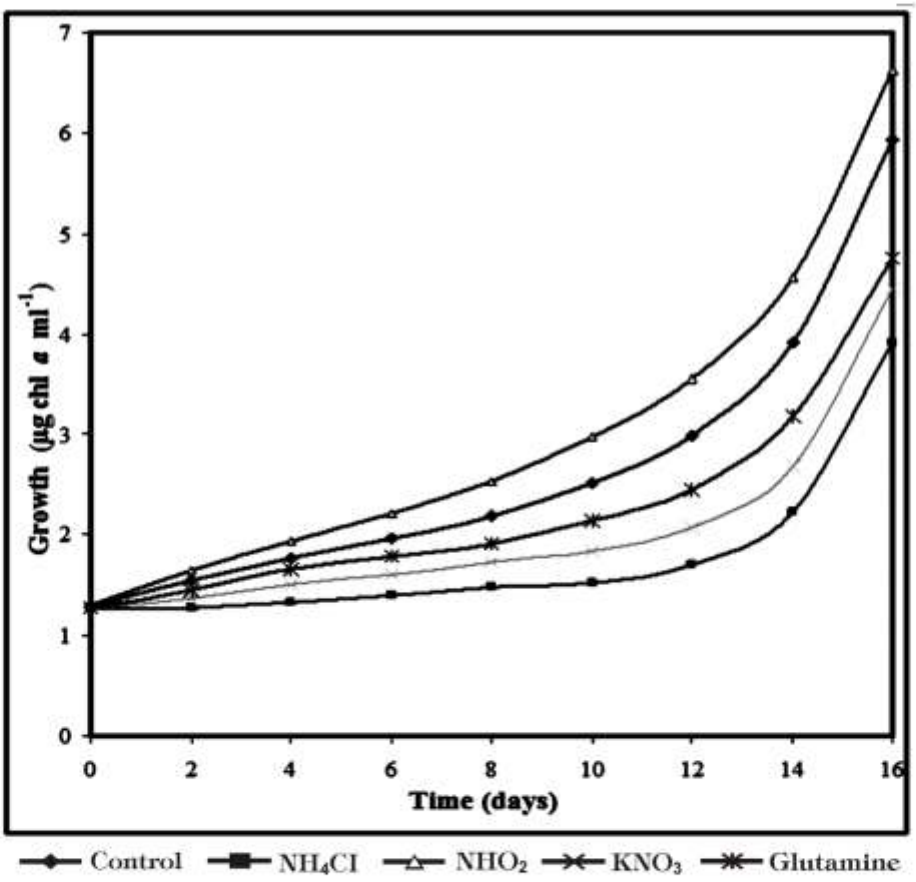

Figure 1: Growth (in terms of chl a) *Anabaena cylindrica* with different nitrogen sources in absence of NaN_3.

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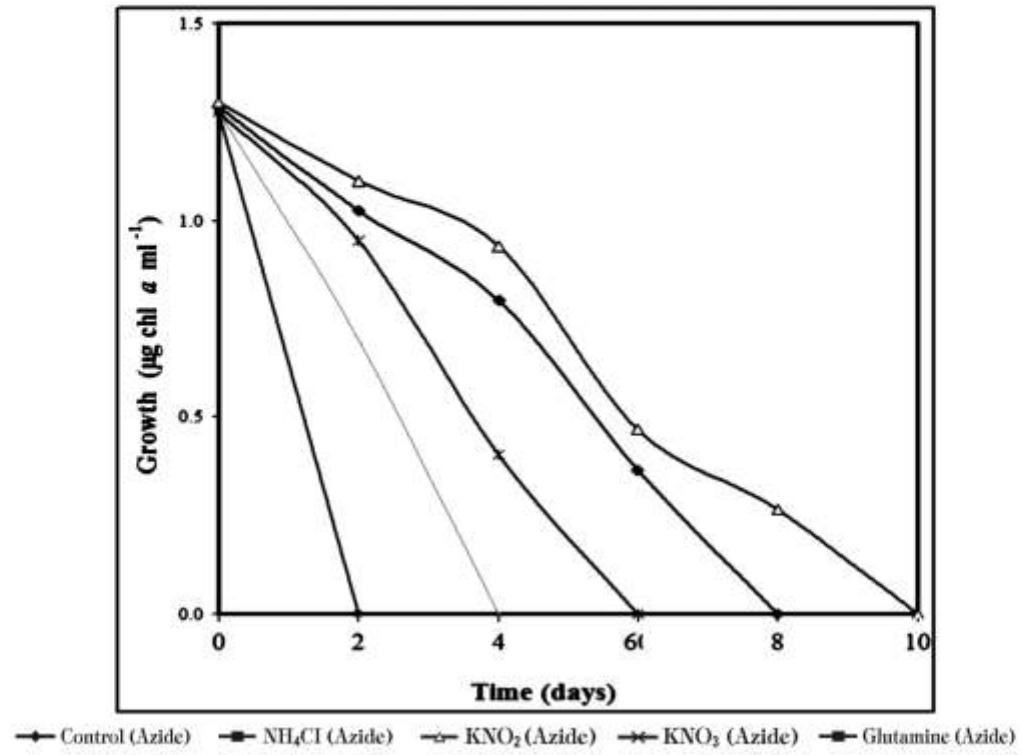

Figure 2: Growth (in terms of chl a) of wild type *Anabaena cylindrica* with different nitrogen sources in response to NaN_3.

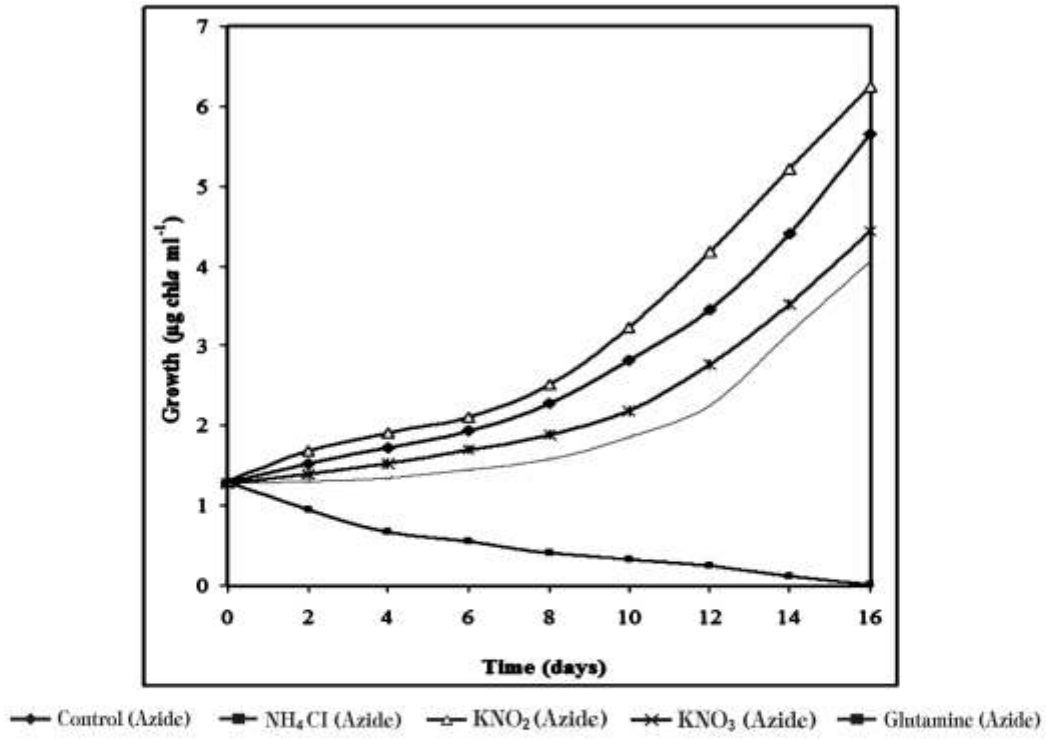

Figure 3: Growth (in terms of chl a) of mutant strain of *Anabaena cylindrica* with different nitrogen sources in response to NaN_3.

Table 1: Heterocyst differentiation (in terms of % heterocyst frequency) of wild type *Anabaena cylindrica* and its mutant counterpart.

Nitrogen sources	Wild type	mutant strain
N_2	6.5	12.5
Glutamine	2.2	4.8
KNO_3	0.0	2.0
KNO_2	0.0	2.8
NH_4Cl	0.0	1.8

References

Bothe, H. 1982. Nitrogen fixation. In: The Biology of Cyanobacteria, Carr, N.G., Whitton, B.A. (eds) *Blackwell Science Publication Oxford*, pp. 87-104.

Chauhan, V. S., Singh, S., Bisen, P. S. 1999a. Development of NaCl resistant mutant strain of the diazotrophic cyanobacterium Anabaena variabilis having derepressed nitrogenase and impaired glutamine synthetase activities. *Physiology and Molecular Biology of Plants*, 5, 33-36.

Chauhan, V.S., Singh, B., Singh, S., Bisen, P.S. 2001. Regulation of sodium influx in the NaCl-resistant (NaCl(r)) mutant strain of the cyanobacterium Anabaena variabilis. *Current Microbiology*, 42(2),100-105.

De, P. K. 1939. The role of blue-green algae in nitrogen fixation in rice fields. *Proceedings of the Royal Society B: Biological Sciences*, 127, 121-139.

Del Campo, F. F., Sanz-Alferez, S., Real-Valcarcel, M., Perez-Silva, G. 1988. Is Nodularia nitrogenase controlled by nitrate? In: *Proceeding of VIth International Symposium on Photosynthetic Prokaryotes,* Mur, L.R. and Burger-Wiesma, T. (eds), Noordwijkerhout, The Netherland, pp. 125.

Hardy, R. W. F., Knight, E. JR. 1967. ATP dependent reduction of azide and HCN by N2 fixing enzymes of Azotobacter vinelandii and Clostridium pasteurianum. *Biochimica et Biophysica Acta*, 139, 69-90.

Richards, D. 2006. Sodium azide based pesticides. *13th Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reduction*, American Pacific Corporation.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., Stanier, R. Y. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology*, 111, 1-61.

Schollhorn, R., Burris, R. H. 1967. Reduction of azide by N2-fixing enzyme. *Proceedings of the National Academy of Sciences* USA, 57, 1317-1323.

Singh, H. N., Singh, H.N. 1978. An azide-resistant mutant of the blue-green alga Nostoc muscorum producing heterocyst and nitrogenase in the presence of fixed inorganic nitrogen source. *Archive of Microbiology*, 119,197-201.

Singh, R. N. 1961. Role of Blue-green Algae in Nitrogen Economy of Indian Agriculture. *Indian Council of Agricultural Research*, New Delhi, India.

Watanabe, A. 1956. The effect of atmospheric nitrogen-fixing blue-green algae on yeild of rice. *Botany Magazine*, 69, 530-535.

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