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Influence of Low pH Stress on Growth, Specific Biochemical Parameters and Antioxidants amongst Selected *Nostoc* Strains

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A B S T R A C T

The present study focused on the influence of low pH on growth, specific biochemical parameters and antioxidants amongst *Nostoc* strains grown under control (pH 7.0) and low pH (pH 4.5) medium. Cell dry weight, chlorophyll content, total soluble proteins and extracellular ammonia release reduced due to low pH stress of growing media compared to control grown cultures. Nitrogenase activity was reduced in two and increased in remaining strains due to low pH. Proline content increased whereas glycerol decreased in all low pH tolerant *Nostoc* strains whereas glycine betaine and lipid peroxidation depicted a variable response.

Introduction

Cyanobacteria are a group of cosmopolitan prokaryotes, which are found in diverse ecological niche including soil, rocks, fresh water and even in salt water (Hoffmann, 1989; Kaushik, 1994). Most of the research work undertaken has focussed on species of *Nostoc*, *Anabaena*, *Tolypothrix*, *Aulosira*, *Cylindrospermum*, *Scytonema* and *Westiellopsis* which are widespread in Indian rice field soils and are known to contribute significantly to the soil fertility (Venkataraman, 1981). Amongst different soil factors, soil pH is particularly important and directly affects cyanobacterial distribution as well as their abundance (Sardeshpande and Goyal, 1981). Under laboratory conditions,

these have generally been reported to prefer neutral to slightly alkaline medium for optimum growth and are normally absent at pH values below 4 or 5 (Gerloff *et al.,* 1952; Kratz *et al.,* 1955). A phytoplankton survey of 10 lakes in Bavarian Forest as well as the lignite mining districts of Bavaria (Upper Palatine) and Lusatia, covering a pH gradient from 8.0 to 2.8, demonstrated that acid-tolerant cyanobacteria do exist (Steinberg *et al.,* 1998). Low pH stress is a potential abiotic stress negatively affecting the growth, survival, pigmentation, protein profiles, membrane structures and biological nitrogen fixation process in cyanobacteria. In 2006, Tandeau de Marsac and Houmard reported that, to survive in extreme or variable environments, cyanobacteria have developed specific regulatory systems in addition to more general mechanisms that are equivalent to those found in other prokaryotes or photosynthetic bacteria. There is limited information on low pH tolerance mechanism in terms of growth and biochemical attributes, however, studies have been conducted in relation to different abiotic stresses like osmotic, salinity, organic, water and UV-radiation. In view of this, the aim of the present work was to study the effect of low pH on growth, total soluble proteins, extracellular ammonia release, nitrogenase activity and antioxidants in selected *Nostoc* strains isolated from low pH soils of India, against fresh water isolate from IARI rice field.

Materials and Methods

Cyanobacterial strains and cultural conditions

Low pH tolerant *Nostoc* strains (Ns1, Ns2, Ns3, Ns4) isolated from acidic soils of India as well as fresh water strain *Nostoc punctiforme* (CCC No. 672, Ns5) which was an isolate from IARI rice field, were procured from the culture collection of CCUBGA, ICAR-IARI. These strains were grown and maintained in BG-11 (N deficient) medium at 28±2˚C temperature under photoperiod of 16:8 hours light and dark cycle with light intensity of 52-55µmole photon $m^{-1}s^{-1}$ in culture room. The pH of medium for Ns1 to Ns4 was maintained at 7.0 (control) and 4.5 (low pH) under two sets of experiment. Low pH was maintained with 0.1M citrate buffer (pH 3.1) comprising 0.1M Citric acid monohydrate and 0.1M Trisodium citrate dihyrate after filter sterilization. *Nostoc punctiforme* (Ns5) was grown and maintained at pH 7.0 only as it did not tolerate low pH medium. Known volumes of cyanobacterial suspension grown under control (pH 7.0) and low pH (pH 4.5) medium was used during exponential phase of growth (14^{th} day) for estimation of growth, specific biochemical attributes and antioxidants.

Cell dry weight (CDW, $mgml^{-1}$) was determined gravimetrically using a known volume of cyanobacterial suspension by centrifugation at 5000g for 10 min. The washed and harvested pellet was dried at 60˚C temperature till constant weight was achieved (Sorokin 1945). The chlorophyll content (μgml^{-1}) was estimated in methanolic extract with absorbance measured at 650 and 665nm (Litchtenthaler and Buschman, 2001). Total soluble proteins were measured at 650 nm spectrophotometrically following the method of Lowry *et al.,* (1951). Phenol hypochlorite method was used to estimate extracellular ammonia (µmole NH_4^+ ml^{-1}, Solorzano1969). Nitrogenase activity was measured as acetylene reducing activity following the method of Hardy *et al*., (1968). Proline content (μgml^{-1}) in cyanobacterial homogenate was determined spectrophotometrically according to the method of Bates *et al.,* (1973). Modified procedures developed by Lambert and Neish (1950) and that of Grieve and Grattan (1983) were used to study glycerol ($\mu g\ ml^{-1}$) and glycine betaine content

(µg ml^{-1}). Concentration of malondialdehyde was measured for lipid peroxidation potential (µg ml^{-1}, Heath & Packer 1968).

For statistical analyses, the triplicate set of data for the various parameters evaluated were subjected to ANOVA (analysis of variance) and the software Statistical Package for Social Sciences (SPSS Version 16.0) was used for calculating SE, SD and CD.

Results and Discussion

Comparative cell dry weight (mg ml^{-1}) as well as chlorophyll content decreased in Ns1, Ns2, Ns3 and Ns4 under low pH growing conditions as compared to control, however, fresh water strain, Ns5 did not tolerate low pH stress and no growth was observed. The percent decrease in the cell dry weight varied from a lowest of 12% in Ns3 to the highest of 25% by Ns1 and the treatment effect was observed to be significant in all. On the other hand, percent decrease in chlorophyll content ranged from a lowest of 17% in Ns3 to the highest of 45% in Ns1 (Table 2). Cell dry weight under control grown conditions showed a highest of 1.90 mg ml^{-1} in Ns1 to the lowest of 1.19 mg ml^{-1} in Ns3. The other two strains showed cell dry weight of 1.77 mg ml^{-1} (Ns2) and 1.1.66 mg ml^{-1} (Ns4) respectively. However, under low pH grown cultures, the range in cell dry weight was from a highest of 1.51 mg ml^{-1} to the lowest of 1.05 mg ml^{-1} in Ns2 and Ns3 while strains Ns1 and Ns2 depicted the cell dry weight of 1.43 mg ml^{-1} and 1.51 mg ml^{-1} respectively. The fresh water strain showed cell dry weight of 1.32 mg ml^{-1} under normal pH of 7.0 (Table 1). *Nostoc* strains which were isolated from low pH soils of India exhibited growth at pH of 4.5 which is in accordance as reported by Rai and Rajsekhar (1989), which showed the growth of cyanobacteria strains at pH 6.3. Earlier study related to pH effect on the growth of cyanobacteria has revealed that the pH between 7.4 and 8.0 is optimum (Rippka *et al.*, 1979; Bano and Siddiqui, 2004). The pH of the medium determines the solubility of CO_2 and minerals in the medium, which in turn can directly or indirectly influence metabolism of these organisms. The pH tolerance has been reported by Venkatraman (1972) and these organisms can grow at a pH range of 6.5 to 10 (Nagle *et al.*, 2010). However, some strains have been reported to grow at a pH as low as 3.5 (Aiyer *et al.*, 1965). In cultures grown under control conditions, strain Ns1 showed highest chlorophyll content of 18.70 µg ml^{-1} whereas strain Ns4 showed lowest chlorophyll content of 12.66 µg ml^{-1}, the other two strains Ns2 and Ns3 showed the chlorophyll content of 13.00 µg ml^{-1} and 15.55 µg ml^{-1} respectively. The fresh water isolate Ns5 showed chlorophyll content of 16.76µg ml^{-1}. However, due to low pH, chlorophyll content was ranged from highest of 12.91 µg ml^{-1} (Ns3) to the lowest of 7.58 µg ml^{-1} (Ns2) while in Ns1 and Ns4 it was 10.22 µg ml^{-1} and 9.01 µg ml^{-1} (Table 1). The reduction in chlorophyll content under low pH stress condition may be due to inhibition of chlorophyll biosynthesis by affecting important enzymes like, α-aminolevulinic acid dehydrogenase and protochlorophyllide reductase, involved in pigment synthesis (Ouzounidou 1995). Huang *et al.*, (2002) also reported that low pH of the medium resulted in the decrease of growth and pigmentation in cyanobacterium *Synechocystis sp.* strain PCC 6308.

Total soluble proteins also decreased due to low pH stress in *Nostoc* strains (Ns1, Ns2, Ns3 and Ns4) as compared to normal pH growing conditions. The fresh water isolate, Ns5 showed the total soluble proteins of 0.71 mg ml^{-1} under control grown conditions. Percent reduction in total soluble proteins was observed to be in the range of 14 % to 24 % by Ns4 and Ns2 due to low pH stress (Table 2). The total soluble proteins were highest

(0.93 mg ml^{-1}) in Ns1 and lowest (0.69 mg ml^{-1}) in Ns4 while Ns2 and Ns3 depicted a total soluble proteins content 0.78 mg ml^{-1} and 0.80 mg ml^{-1} at pH 7.0. At pH 4.5, Ns1 showed highest (0.73 mg ml^{-1}) followed by 0.69 mg ml^{-1} (Ns3), 0.59 mg ml^{-1} (Ns4) and Ns2 showed lowest (0.59 mg ml^{-1}) total soluble proteins (Table 1). Extracellular ammonia release is a very important attribute of heterocystous cyanobacteria and ranged from a highest of 275.20 µmole NH_4^+ ml^{-1} (Ns1) to lowest of 68.90 µmole NH_4^+ ml^{-1} (Ns4), while the other two strains Ns2 and Ns3 depicted 191.10 µmole NH_4^+ ml^{-1} and 87.70 µmole NH_4^+ ml^{-1} under control grown conditions. When these cultures were grown under low pH medium, the extracellular ammonia release ranged from 165.10 µmole NH_4^+ ml^{-1} in Ns1 to 30.70 µmole NH_4^+ ml^{-1} in Ns4 while, the Strains Ns2 and Ns3 depicted the extracellular ammonia release of 75.10 µmole NH_4^+ ml^{-1} and 38.80 µmole NH_4^+ ml^{-1} respectively. Fresh water isolate showed an extracellular ammonia release of 103.40 µmole NH_4^+ ml^{-1} and percent decrease in extracellular ammonia release varied as a highest of 61% to the lowest of 40% in Ns2 and Ns1 due to low pH stress in the growing medium (Table 2). The reduction in extracellular ammonia release could be due to enhanced glutamine synthetase activity or reduced nitrogenase activity under low pH stress condition.

Fresh water strain does not tolerate low pH stress growing condition as cyanobacteria have been reported to prefer alkaline condition for their growth which suggests that there is probably an acid barrier which these organisms are not able to overcome, hence, this group of algae is excluded from low pH environment. However, in other strains of *Nostoc* (Ns1, Ns2, Ns3, Ns4) there was a significant decrease observed in cell dry weight, chlorophyll content, total soluble proteins and extracellular ammonia release due to low pH stress which could be as a result of lack of control over internal pH resulting in growth limitation (Padan *et al.,* 1981; Booth 1985; Padan and Schuldiner 1987). At low pH, cells spend energy for maintenance of internal pH necessary for important cell functions (Raven and Lucas 1985). Low pH tolerance shown by cyanobacteria suggests that these organisms can adapt to variable pH conditions (Burja *et al.,* 2002). However, the growth rate of diatoms was not affected by pH range of 7.4 to 8.2 and it was significantly lower at pH of 6.8. Highest nitrogenase activity of 1.96 nmole C_2H_4 $ml^{-1}h^{-1}$ was exhibited by Ns2 followed by 0.30 nmole C_2H_4 $ml^{-1}h^{-1}$ in Ns1, 0.32 nmole C_2H_4 $ml^{-1}h^{-1}$ in Ns3 and the lowest of 0.28 nmole C_2H_4 $ml^{-1}h^{-1}$ was shown in Ns4 under control grown culture conditions. When the cultures were grown under low pH stress medium, nitrogenase activity dropped to a highest of 0.83 nmole C_2H_4 $ml^{-1}h^{-1}$ in Ns3 and lowest of 0.27 nmole C_2H_4 $ml^{-1}h^{-1}$ in Ns1. The other two strains, Ns2 and Ns4 showed the nitrogenase activity of 0.64 nmole C_2H_4 $ml^{-1}h^{-1}$ and 0.64 nmole C_2H_4 $ml^{-1}h^{-1}$ respectively (Table 1). Low pH stress condition reduced the nitrogenase activity by 10% and 67% in *Nostoc* strains Ns1 and Ns2, however, it increased in Ns3 and Ns4 by 159% and 139% and fresh water isolate showed nitrogenase activity of 1.73 nmole C_2H_4 $ml^{-1}h^{-1}$ (Table 2). There was a strain variability recorded in terms of expression of nitrogenase activity due to low pH condition. Comparative evaluation of selected parameters amongst *Nostoc* strains grown under control (pH 7.0) and low pH (pH 4.5) conditions depicted that *Nostoc* strain Ns1, an isolate from Alipurduar (West Bengal), India showed maximum cell dry weight, chlorophyll content, total soluble proteins and extracellular ammonia release while Ns4 (an isolate from Mokokchung, Nagaland soil) showed lowest chlorophyll, total soluble proteins and extracellular ammonia release under control (pH 7.0)

grown conditions. The cell dry weight was lowest in Ns3, which was an isolate from Ernakulum Kerala soil. When these cultures were grown under low pH medium, Ns1 again depicted highest total soluble proteins and extracellular ammonia release, whereas, Ns2 showed highest cell dry weight and Ns3 exhibited maximum chlorophyll content. On the other hand Ns2 showed lowest chlorophyll content and total soluble proteins, whereas, cell dry weight was lowest in Ns3 and extracellular ammonia release was lowest in Ns4.

Results calculated for non-enzymatic antioxidants namely proline, glycerol, glycine betaine and lipid peroxidation indicated a variable behaviour by *Nostoc* strains grown under control and low pH conditions. The low pH stress enhanced selected antioxidants like proline, lipid peroxidation and glycine betaine, however, a reverse trend was shown in terms of glycerol accumulation. Proline content increased in *Nostoc* strains under pH 4.5 as compared to control grown cultures. Under low pH stress, the highest proline content of 132.80 µg ml^{-1} was recorded in Ns1 and the lowest of 76.68 µg ml^{-1} was shown by Ns3. The other two strains showed proline content of 102.63 µg ml^{-1} (Ns2) and 101.47 µg ml^{-1} (Ns4) respectively. When *Nostoc* strains were grown at control pH medium, the proline content of 86.38 µg ml^{-1} was highest in Ns2 followed by a proline content of 75.54 µg ml^{-1} (Ns1), 64.76 µg ml^{-1} (Ns4) with the lowest of 61.70 µg ml^{-1} (Ns3). Fresh water isolate showed a proline content of 64.34 µg ml^{-1} which was similar to the proline content depicted by Ns4 (Table 3).

Table.1 Comparative Cell Dry Weight (CDW, mg ml^{-1}), chlorophyll (µg ml^{-1}), total soluble proteins (TSP, $mgml^{-1}$), extracellular ammonia release (EAR, µmole ml^{-1}) and nitrogenase activity (nmoles C_2H_4 ml^{-1} h^{-1}) amongst selected *Nostoc* strains grown under control (pH-7.0) and low pH (pH-4.5) conditions (Mean ± SD; n=3)

Strains*/ Treatments†	CDW	Chlorophyll	TSP	EAR	Nitrogenase≠
Ns1 C1	1.90 ± 0.076^{a}	18.70 ± 1.108^{a}	0.93 ± 0.055^{a}	275.20 ± 3.995^{a}	0.30 ± 0.003^{e}
T1	1.43 ± 0.053^{de}	10.22 ± 2.656^{d}	0.73 ± 0.013^{bc}	165.10 ± 1.212^{c}	0.27 ± 0.006^{e}
Ns2 C2	1.77 ± 0.020^{b}	13.00 ± 0.847^{c}	0.78 ± 0.029^{b}	191.10 ± 2.163^{b}	1.96 ± 0.055^{a}
T2	1.51 ± 0.102^{d}	7.58 ± 1.589^{e}	0.59 ± 0.033^{d}	75.10 ± 1.539^{f}	0.64 ± 0.001^{d}
Ns3 C3	1.19 ± 0.068^{g}	15.55 ± 0.674^{b}	0.80 ± 0.016^{b}	87.70 ± 1.652^{e}	0.32 ± 0.013^{e}
T3	1.05 ± 0.080^{h}	12.91 ± 0.201^{c}	0.69 ± 0.026^{c}	38.80 ± 0.458^{h}	0.83 ± 0.003^{c}
Ns4 C4	1.66 ± 0.054^{c}	12.66 ± 0.117^{c}	0.69 ± 0.064^{c}	68.90 ± 2.272^{g}	0.28 ± 0.011^{e}
T4	1.39 ± 0.017^{ef}	9.01 ± 0.063^{de}	0.59 ± 0.027^{d}	30.70 ± 1.353^{i}	0.67 ± 0.010^{d}
Ns5 C5	1.32 ± 0.024^{f}	16.76 ± 0.734^{ab}	0.71 ± 0.026^{c}	103.40 ± 2.551^{d}	1.73 ± 0.041^{b}
T5	ND#	ND	ND	ND	ND
SEm (±)	**0.037**	**0.617**	**0.021**	**1.285**	**0.015**
CD (0.05%)	**0.112**	**1.850**	**0.063**	**3.854**	**0.044**

* *Nostoc* strains (Ns1, Ns2, Ns3, Ns4, Ns5)

† Treatments: Control (C1, C2, C3, C4, C5); low pH (T1, T2, T3, T4, T5)

≠ Acetylene reducing activity

Different superscripts in the same column represent significant differences between samples ($p< 0.05$)

ND- Not detected

Table.2 Percent change in cell dry weight (CDW), chlorophyll, total soluble proteins (TSP), extracellular ammonia release (EAR) and nitrogenase activity amongst selected *Nostoc* strains under low pH stress condition as compared to control

Strains	CDW	Chlorophyll	TSP	EAR	Nitrogenase
Ns1	25% ↓	45% ↓	22% ↓	40% ↓	10% ↓
Ns2	15% ↓	42% ↓	24% ↓	61% ↓	67% ↓
Ns3	12% ↓	17% ↓	14% ↓	56% ↓	159% ↑
Ns4	16% ↓	29% ↓	14% ↓	55% ↓	139% ↑

*Arrows denotes percent increase (↑) and decrease (↓) in specific parameter

Table.3 Comparative proline (µg mg^{-1}), glycerol (µg mg^{-1}), glycine betaine (µg mg^{-1}) and lipid peroxidation (µg mg^{-1}) amongst selected *Nostoc* strains grown under control (pH- 7.0) and low pH (pH- 4.5) condition (Mean ± SD; n=3)

Strains*/ Treatments†		Proline	Glycerol	Glycine betaine	Lipid peroxidation
Ns1	C1	75.94 ± 1.279^{d}	34.10 ± 1.463^{d}	200.76 ± 2.001^{a}	4.19 ± 0.047^{b}
	T1	132.80 ± 2.558^{a}	28.79 ± 0.770^{e}	109.22 ± 1.044^{g}	3.48 ± 0.068^{c}
Ns2	C2	86.38 ± 0.483^{c}	46.60 ± 1.016^{b}	145.55 ± 0.688^{f}	3.24 ± 0.094^{d}
	T2	102.63 ± 1.675^{b}	25.74 ± 0.438^{f}	162.18 ± 0.168^{c}	5.96 ± 0.060^{a}
Ns3	C3	61.70 ± 0.796^{f}	34.28 ± 0.194^{d}	151.18 ± 1.529^{e}	2.18 ± 0.013^{g}
	T3	76.68 ± 0.633^{d}	14.83 ± 0.848^{h}	200.73 ± 2.988^{a}	2.85 ± 0.073^{e}
Ns4	C4	64.76 ± 0.659^{e}	42.19 ± 0.308^{e}	185.35 ± 0.175^{b}	2.51 ± 0.013^{f}
	T4	101.47 ± 3.186^{b}	21.09 ± 1.272^{g}	155.41 ± 1.379^{d}	2.85 ± 0.026^{e}
Ns5	C5	64.34 ± 1.096^{ef}	48.76 ± 0.321^{a}	162.04 ± 0.679^{c}	3.50 ± 0.045^{c}
	T5	ND#	ND	ND	ND
SEm (±)		**0.996**	**0.501**	**0.789**	**0.032**
CD (0.05%)		**2.987**	**1.501**	**2.366**	**0.097**

* *Nostoc* strains (Ns1, Ns2, Ns3, Ns4, Ns5)

† Treatments: control (C1, C2, C3, C4, C5); low pH (T1, T2, T3, T4, T5)

Different superscripts in the same column represent significant differences between samples ($p < 0.05$)

ND- Not detected

Table.4 Percent change in proline, glycerol, glycine betaine and lipid peroxidation amongst selected *Nostoc* strains under low pH stress condition as compared to control

Strains	Proline	Glycerol	Glycine betaine	Lipid peroxidation
Ns1	75% ↑	16% ↓	46% ↓	17% ↓
Ns2	19% ↑	45% ↓	11% ↑	84% ↑
Ns3	24% ↑	57% ↓	33% ↑	31% ↑
Ns4	57% ↑	50% ↓	16% ↑	14% ↑

*Arrows denotes percent increase (↑) and decrease (↓) in specific parameter

Glycerol content also decreased due to low pH stress compared to control and the percent decrease varied from 16% in Ns1 to 57% in Ns3 (Table 4). Fresh water isolate (Ns5) showed a highest glycerol content of 48.76 µg ml^{-1}, followed by glycerol content of 46.60 µg ml^{-1} (Ns2), 42.19 µg ml^{-1} (Ns4), 34.28 µg ml^{-1} (Ns3) with the lowest of 34.10 µg ml^{-1} (Ns1) under control grown conditions. However at low pH, the glycerol content was highest (28.79 µg ml^{-1}) in Ns1 and lowest (14.83 µg ml^{-1}) in Ns3, while the other two strains, Ns2 and Ns4 depicted the glycerol content of 25.74 µg ml^{-1} and 21.09 µg ml^{-1} respectively (Table 3). Glycine betaine was highest (200.76 µg ml^{-1}) in Ns1 followed by 151.18 µg ml^{-1} in Ns3, 185.35 µg ml^{-1} in Ns4 and Ns2 showed a lowest glycine betaine of 145.55 µg ml^{-1} at pH 7.0 whereas, fresh water isolate depicted a glycine betaine level of 162.04 µg ml^{-1} which was *at par* with the glycine betaine content of 162.18 µg ml^{-1} under low pH stress by Ns2. Under pH stress, the glycine betaine was highest (200.73 µg ml^{-1}) in Ns3 whereas Ns1 showed lowest (109.22 µg ml^{-1}) glycine betaine content while, the other two strains, Ns2 and Ns4 showed 162.18 µg ml^{-1} and 155.41 µg ml^{-1} of glycine betaine content respectively (Table 3). *Nostoc* strains exhibited a variable behaviour in terms of glycine betaine which increased by 11% and 33% in Ns2 and Ns3 due to low pH stress, whereas a decrease of 16% and 46% was recorded by Ns4 and Ns1 (Table 4). Lipid peroxidation also depicted a variability in terms of response towards low pH stress *vis-à-vis* control grown cultures with the highest of 4.19 µg ml^{-1} recorded by Ns1 and lowest of 2.18 µg ml^{-1} recorded by Ns3 under pH 7.0. The fresh water isolate showed lipid peroxidation of 3.50 µg ml^{-1}. At low pH, Ns2 showed highest (5.96 µg ml^{-1}) lipid peroxidation followed by Ns1 (3.48 µg ml^{-1}), Ns3 (2.85 µg ml^{-1}) and Ns4 (2.83 µg ml^{-1}) (Table 3). Lipid peroxidation was more or less similar in Ns3 and Ns4 when the cultures were grown under control (pH 7.0) and/or low pH stress condition. Lipid peroxidation decreased by 17% in Ns1 and increased by 84%, 31% and 14% in Ns2, Ns3 and Ns4 due to low pH stress as compared to control (Table 4). High degree of lipid peroxidation has been reported in *Synechococcus* and *Nostoc muscorum* (Rehman *et al.,* 2011). Stress induced enhancement in these parameters is supported by the reports of Zeesan and Prasad (2009) and Sunderam *et al.,* (2011). The stress and the resistance is governed through modulation of antioxidant enzymes as well as compounds like proline, glycine betaine, glutathione and ascorbate and their increased malondialdehyde levels. Increased level of these antioxidants under stress condition is indicative of a correlation between free radical generation and proline accumulation (Zeesan and Prasad 2009). This is in agreement with other reports on *Spirulina platensis* and *Westeillopsis prolifica* (Choudhary *et al.*2007). The cyanobacteria can counteract the toxic effect of abiotic stress induced free radicals by increasing antioxidants defense mechanisms.

Under control conditions of growth, proline content was highest in Ns 2, whereas glycerol was maximum in Ns5 and Ns1 showed maximum glycine betaine and lipid peroxidation. However, the proline and lipid peroxidation was lowest in Ns3, while glycerol and glycine betaine were lowest in Ns1 and Ns2. Highest proline content and glycerol was depicted by Ns1 whereas glycine betaine and lipid peroxidation were maximum in Ns3 and Ns2 when cultures were grown under low pH stress. With pH stress proline and lipid peroxidation were lowest in Ns4 whereas, glycerol was lowest in Ns3 and glycine betaine was lowest in Ns1.

In conclusions, comparative studies undertaken amongst *Nostoc* strains indicated

that low pH condition in the growing medium reduced growth in terms of cell dry weight, chlorophyll content, extracellular ammonia release and total soluble proteins. The influence of low pH on nitrogenise activity was variable. Low pH stress increased proline content and increased glycerol, however, its influence on other parameters like glycine betaine and lipid peroxidation was variable. The study clearly indicated the differential behaviour of *Nostoc* strains in terms of selected parameters due to low pH stress situations and such strains can be further used to understand the in-depth mechanisms underlying low pH tolerance amongst cyanobacteria.

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