

Cytomorphological and nitrogen metabolic enzyme analysis of psychrophilic and mesophilic *Nostoc* sp.: a comparative outlook

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Abstract Cyanobacterial diazotrophs play a significant role in environmental nitrogen economy despite their habitat either tropical or polar. However, the phenomenon by which it copes with temperature induced stress is poorly understood. Temperature response study of psychrophilic and mesophilic *Nostoc* strains explores their adaptive mechanisms. The selected psychrophilic and mesophilic strains were confirmed as *Nostoc punctiforme* and *Nostoc calcicola* respectively, by ultrastructure and 16S rDNA phylogeny. The psychrophilic strain has extensive glycolipid and polysaccharide sheath along with characteristic deposition of cyanophycin, polyhydroxybutyrate granules, and carboxysomes. This is possibly an adaptive strategy exhibited to withstand the freezing temperature and high intense of ultraviolet rays. The biomass measured in terms of dry weight, protein, and chlorophyll indicated a temperature dependant shift in both the psychrophilic and mesophilic strains and attained maximum growth in their respective temperature niches. At low temperature, psychrophilic organism exhibited nitrogenase activity, while mesophilic strains did not. The maximum glutamine synthetase activity was observed at 4 °C for psychrophilic and 37 °C for mesophilic strains. Activity at 4 °C in psychrophilic strains revealed their energetic mechanism even

at low temperature. The nitrate and nitrite reductase of both psychrophilic and mesophilic strains showed maximum activity at 37 °C denoting their similar nitrogen assimilating mechanisms for combined nitrogen utilization. The activity studies of nitrogen fixation/assimilation enzymes have differential effects at varying temperatures, which provide valuable insights of physiological contribution and role of *Nostoc* strains in the biological nitrogen cycle.

Keywords Temperature stress · Nitrate reductase (NR) · Nitrite reductase (NIR) · Glutamine synthetase (GS) · Nitrogenase

Introduction

Cyanobacteria exist in various ecological niches ranging from polar to desert climate zones because of their versatility, diverse metabolism, environmental plasticity, and structural conservation (Premanandh et al. 2009). Thus, the cyanobacteria comprise of psychrophilic, psychrotolerant, mesophilic, and thermophilic strains signifying wide thermal adaptability. Temperature is one of the best decisive regulatory factors for the membrane fluidity, enzyme activity, and electron chain transfer, thereby influencing the process of nutrient uptake, growth, and photosynthesis (Staeher and Jensen 2006). Cyanobacterial diazotrophs play a significant role in nutrient cycling by their inherent ability to fix atmospheric nitrogen and carbon (Saha et al. 2007; Prabaharan et al. 2010). Cyanobacteria fixes atmospheric nitrogen in different thermal extremes, including Antarctic, where the temperature is always below 0 °C (Priscu et al. 1998), in an arid desert (Winckelmann et al. 2015) and in hot spring where the temperature ranges from 55 to 70 °C (Amarouche-Yala et al. 2014).

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Cold ecosystem prevails in more than three-quarters of the earth's surface, including the ocean depths, polar, and alpine regions. Due to the persistent freezing temperature, high UV irradiance and the extreme fluctuation in nutrient cycle, relatively very limited biodiversity, exist in these zones. In Arctic and Antarctic climates, cyanobacteria appear first and dominate the microflora during the summer period in both environments. Notoriously, cyanobacteria could grow efficiently under low nutrient conditions (Mishra and Mukherji 2012). Of the different nutrients, nitrogen is found to be limiting in the Arctic ecosystem (Quesada et al. 1999). Under nitrogen limiting conditions in these regions, diazotrophic cyanobacteria especially *Nostoc* genera predominate and contribute to a large extent towards the carbon and nitrogen economy (Komarek et al. 2012; Viderova et al. 2011). The factors that affect the terrestrial nitrogen fixation in a polar environment are soil moisture and temperature (Stewart et al. 2011). Hence, the study was planned to compare and assess the temperature influence on growth and their nitrogen fixation/assimilation ability of a *Nostoc* sp. of polar Arctic and mesophilic isolates.

Materials and methods

Source of the organisms

All the three *Nostoc* strains are chosen from National Repository for Microalgae and Cyanobacteria (NRMC), Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The psychrophilic freshwater isolate *Nostoc punctiforme* BDU ARC 10101 is from Ny-Alesund, Svalbard and maintained at 3 ± 2 °C under a constant white fluorescent light of $20 \mu\text{mol photons m}^{-2} \text{S}^{-1}$. The other two marine mesophilic *Nostoc calcicola* BDU 40302 and BDU 180601 isolates are from Gulf of Mannar and Gulf of Kutch, India, respectively, and maintained at 25 ± 2 °C under a constant white fluorescent light of $20 \mu\text{mol photons m}^{-2} \text{S}^{-1}$.

Experimental conditions

Marine mesophilic and freshwater psychrophilic *Nostoc* strains were inoculated into nitrogen free ASN III and BG 11 medium, respectively (Rippka et al. 1979) and incubated at different temperatures viz., 4, 15, 25, and 37 °C illuminated with white fluorescent light of $20 \mu\text{mol photons m}^{-2} \text{S}^{-1}$ at 14/10 h light/dark cycle for a period of 7 days. For performing enzyme assays, mesophilic and psychrophilic strains were incubated overnight in their respective nitrogen supplemented media illuminated with continuous light of $20 \mu\text{mol photons m}^{-2} \text{S}^{-1}$.

Microscopic analysis

Light microscopy

Light microscopic observation of the selected strains was carried out using Leica inverted microscope DMI 3000B (Leica, Germany) and images were documented with Leica digital camera DFC 425C and processed with the Leica application suite software.

Ultra structure

Freshly harvested cells of the three strains were fixed in 3% glutaraldehyde separately. The cells were washed with sodium cacodylate buffer (0.1 M; pH 7.4) and post fixed in 1% osmium tetroxide. Then, the cells were washed in the same buffer, dehydrated using graded alcohol in the ascending series (50–100%), and finally cleared by propylene oxide. Samples were infiltrated by propylene oxide and epoxy resin and embedded onto siliconized rubber molded with epoxy resin. The embedded mould was allowed to polymerize by incubating at 60 °C for 48 h. Ultrathin sections were prepared by ultramicrotomy (Leica ultra cut UCT) and loaded onto a copper-coated grid. Sections contrasted with uranyl acetate and Reynold's lead citrate solution (Diestra et al. 2007) were examined under the transmission electron microscope (TEM) (Philips Tecnai TR spirit transmission electron microscope, The Netherlands), and the microphotographs were documented.

DNA extraction and amplification

Total genomic DNA was extracted from the chosen three *Nostoc* strains using the xanthogenate nucleic acid isolation protocol (Tillet and Neilan 2000). To generate the complete sequence of 16S rDNA, the gene was amplified by splitting it into three regions and amplified using a different set of primers. Amplification of I region was performed using 16S-8 forward primer -AGAGTTTGATCCTGGCT CAG and 16S-740 reverse primer -TCTACGCATTT CACCGCTAC described by Seo and Yokota (2003), II region was amplified using the cyanobacterial specific primer CYA-359 forward -GGG GAATTTTCCG CAATGGG and equimolar ratio of CYA-781(A) reverse primer -GACTACTGGGGTATCTAATCCCATT and CYA781(B) reverse primer -GACTACAGGGGTATCT AATCCCTTT as described by Nubel et al. (1997), and the III region was amplified using CYA-359 forward primer -GGGGAATTTTCCGCAATGGG (Nubel et al. 1997) and 16S-1494 reverse primer -GTACGGCTACCTTGTT ACGAC (Tillet et al. 2001). Amplification was carried out

in Eppendorf master cycler Pro S using GoTaq colorless master mix (Promega, US), 0.3 μM of each primer, and 100 ng of template DNA in a 100 μl reaction. The cycling profile encompasses an initial denaturation of 94 $^{\circ}\text{C}$ for 5 min followed by 30 cycles of denaturation at 94 $^{\circ}\text{C}$ for 1 min, annealing (I region: 55.5 $^{\circ}\text{C}$, II region: 60 $^{\circ}\text{C}$, and III region: 56.4 $^{\circ}\text{C}$ for 1 min) and extension at 72 $^{\circ}\text{C}$ for 1 min and a final extension at 72 $^{\circ}\text{C}$ for 7 min. 10 μl of the amplified products subjected to 1.5% agarose gel electrophoresis stained with ethidium bromide were visualized under UV trans-illuminator.

16S rRNA gene sequence analysis

The amplified PCR products were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced at Eurofins Genomics India PVT Ltd, Bangalore, India. All the forward and reverse sequences of three regions of the electropherograms were checked by manual inspection and assembled into a single contiguous sequence of 16S rRNA gene using the software DNA Baser version 3.5.1 (<http://www.dnabaser.com>). Sequences thus obtained were blasted against nucleotide blast of NCBI (<http://blast.ncbi.nlm.nih.gov>) to identify the closely related taxa for sequence comparison. Multiple sequence alignment was performed by Clustal X (Thompson et al. 1997) and the phylogenetic tree was generated using the MEGA software version 6 (Tamura et al. 2013).

Nucleotide sequence accession numbers

Three nucleotide sequences of the psychrophilic *Nostoc punctiforme* BDU ARC 10101 and mesophilic *Nostoc calcicola* BDU 40302 and BDU 180601 strains obtained from this study are submitted to GenBank and their Accession numbers are KC883979, KC883980, and KC883981.

Dry weight estimation

All the three cyanobacterial cultures were centrifuged on the seventh day of growth, washed with distilled water, and dried at 60 $^{\circ}\text{C}$ for at least two concordant values.

Chlorophyll estimation

Chlorophyll *a* was extracted in 80% methanol in the dark. The optical density of the supernatant was read at 663 nm in Cary 100 bio UV–VIS spectrophotometer and estimated following Mac Kinney (1941).

Carotenoid estimation

Carotenoid was extracted in 85% acetone in the dark. After centrifugation, the optical density of the supernatant was read at 450 nm in Cary 100 bio UV–VIS spectrophotometer and the amount was estimated by employing the extinction coefficient of Jensen (1978).

Protein estimation

The total soluble protein content of the extracts was measured as described by Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

Nitrogen fixing/assimilating enzymes

Nitrogenase (ARA, E.C. 1.18.6.1) activity was determined by acetylene reduction assay (ARA) (Stewart et al. 1967) in all the three *Nostoc* strains grown at varied temperatures of 4, 15, 25, and 37 $^{\circ}\text{C}$ in sealed air-tight vials. From the above vials, 10% gas phase was withdrawn and replaced with acetylene. The assay vials were incubated in an illuminated shaker (100 rpm) at respective temperatures under cool white fluorescent light of 20 $\mu\text{mol photons m}^{-2} \text{S}^{-1}$ for an hour. The enzyme activity was stopped by injecting 0.2 ml of 20% trichloroacetic acid (TCA, Sigma Aldrich, USA) to each vial. For the determination of ethylene concentration, 100 μl of gaseous phase was injected into Poropak-T column (oven temperature: 75 $^{\circ}\text{C}$; detector temperature: 120 $^{\circ}\text{C}$; carrier gas–nitrogen at 30 ml min^{-1}) in a Gas chromatograph Clarus 500 (Perkin Elmer, USA) and detected with a flame ionisation detector. Ethylene (Sigma Aldrich, USA) was used as standard. The nitrogenase activity was expressed in terms of nanomoles of ethylene formed $\text{h}^{-1} \text{G}^{-1}$ dryweight.

Glutamine synthetase (GS, E.C.6.3.2.3) assay was performed in toluene permeabilized cyanobacterial pellet at 4 $^{\circ}\text{C}$. The GS activity was estimated in vitro by incubating the clear supernatant with 1 ml of reaction mixture for 30 min in the dark at different temperatures of 4, 15, 25, and 37 $^{\circ}\text{C}$. After stopping the reaction, absorbance was read at 540 nm and the amount of activity was expressed as $\mu\text{g } \gamma\text{-glutamyl hydroxamate formed mg}^{-1} \text{protein}$ (Shapiro and Stadtman 1970). Nitrate reductase (NR, E.C.1.6.6.1) and Nitrite reductase (NIR, E.C.1.7.7.2) enzymes were spectrophotometrically assayed as nitrate reduction with sodium dithionite and reduced methyl viologen as the electron donor (Herrero et al. 1981). The amount of activity was expressed as $\mu\text{g nitrite formed/removed mg}^{-1} \text{protein}$, respectively.

Results and discussion

Cyanobacteria, the oxygen evolving photosynthetic prokaryotes, are ubiquitous in nature and found all over the world encompassing ice continents of Arctic and Antarctic (Vezina and Vincent 1997) to hot spring, where the temperature is near 70 °C (Amarouche-Yala et al. 2014). Freezing temperatures accompanied by freeze–cycles, extreme fluctuation in UV irradiance, and low availability of nitrogen are major problems in polar regions hampering the growth of cyanobacteria (Grzesiak et al. 2015). In spite of drastic climatic conditions, cyanobacteria not only grow in lower temperature, but also play a major role in nitrogen and carbon cycle of this extreme habitat. To understand the nitrogen metabolism of cyanobacteria at psychrophilic temperature, both psychrophilic and mesophilic *Nostoc* strains isolated from their respective regimes were compared for their growth and N₂ fixing/assimilating capabilities in different temperatures.

Morphologically, all the three chosen strains possessed unbranched trichomes, barrel-shaped cells with rounded, or subspherical heterocyst and are designated as *Nostoc* (Desikachary 1959). The polar strain designated as *Nostoc punctiforme* Hariot BDU ARC 10101 is characterized by compactly linked trichomes with colourless sheath, cell length: 4.9 μm, breadth: 4.4 μm with rounded heterocyst (Fig. 1a), while the strain isolated from Gulf of Mannar exhibited long, loosely associated trichomes with a colourless sheath, and cell exhibited length: 4.2 μm,

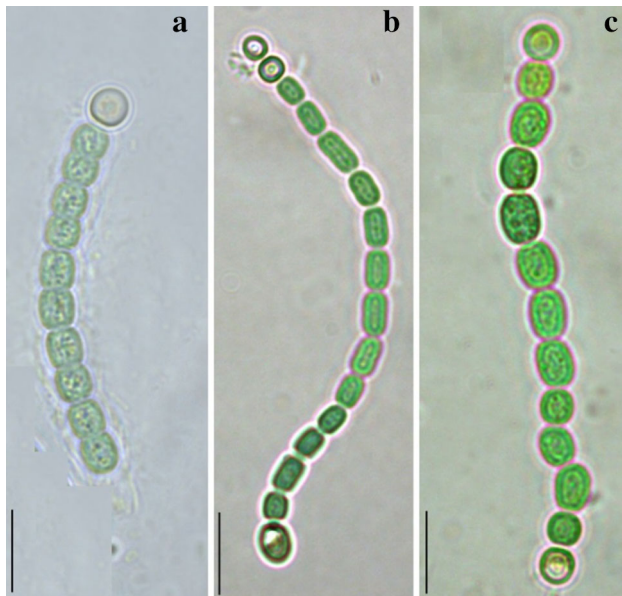
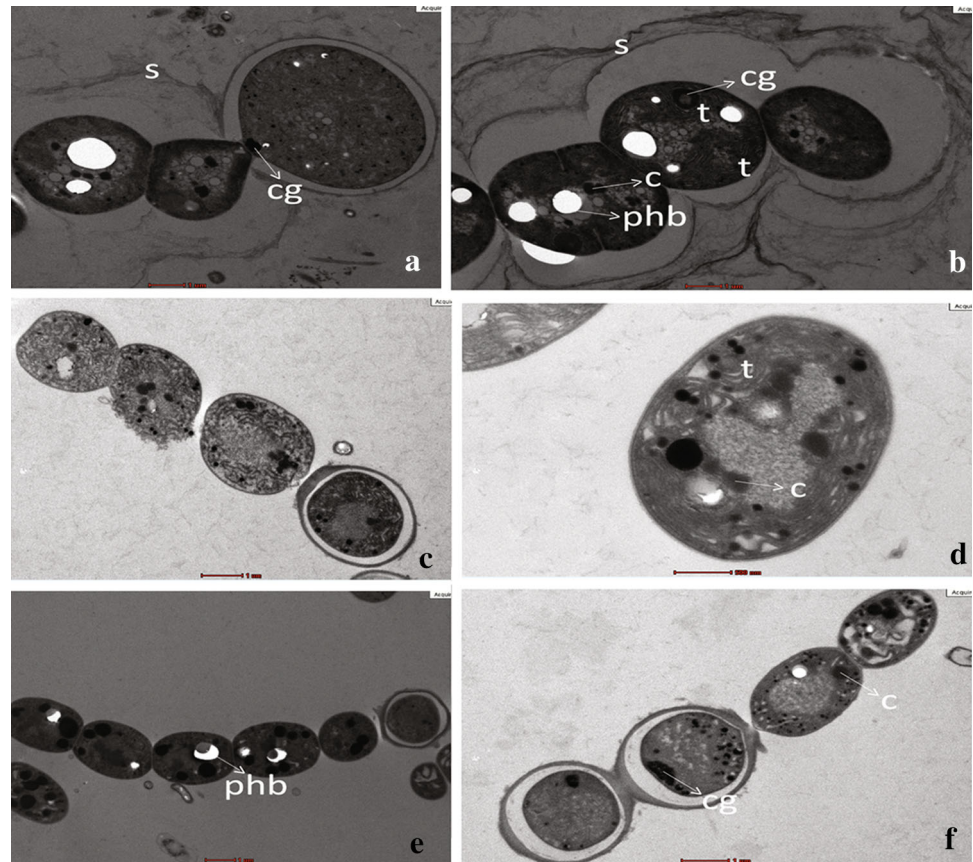


Fig. 1 Bright field photomicrographs **a** *Nostoc punctiforme* Hariot BDU ARC 10101, **b** *Nostoc calcicola* Brebisson BDU 40302, and **c** *Nostoc calcicola* Brebisson BDU 180601 taken at the magnification as indicated by the scale bar (10 μm) in the lower left of the picture

breadth: 2.3 μm, with sub spherical heterocyst is specified as *Nostoc calcicola* Brebisson BDU 40302 (Fig. 1b). The strain isolated from Gulf of Kutch possessed mucilaginous thallus, its trichomes are long, loosely affiliated with a colourless sheath, with cell length: 4.3 μm, breadth: 2.7 μm, and heterocyst sub-spherical is identified as *Nostoc calcicola* Brebisson BDU 180601 (Fig. 1c). Among the three organisms, polar strain possessed slightly larger cells with round heterocyst. On careful scrutiny, the ultra structural images of mesophilic strains *Nostoc calcicola* BDU 40302 (Fig. 2c, d) and *Nostoc calcicola* BDU 180601 (Fig. 2e, f) exhibited the presence of glycolipid and polysaccharide layers external to the normal cell wall. The heterocyst and the neighboring vegetative cells were very loosely connected by a narrow pore channel and also showed detachment from one another in certain junctures. These ultra structural features were analogous with ultra structure of *Nostoc commune* isolated from the building walls of Mayan monuments (Ramirez et al. 2011). Thylakoids of the larger vegetative cells were located peripherally, with whorls pushing through the center. Nucleoid spaces contained trapezoidal carboxysomes. Cyanophycin and polyhydroxybutyrate granules were present in the vegetative cells of *Nostoc calcicola* BDU 180601. In the case of psychrophilic strain *Nostoc punctiforme* BDU ARC 10101 (Fig. 2a, b), many chains of vegetative cells were covered by multilayer sheaths of extensive glycolipid and polysaccharide layer. The junction between the heterocyst and the neighboring vegetative cell is very narrow and this region shows the characteristic deposition of cyanophycin granules. Cells displayed an accumulation of cyanophycin, polyhydroxybutyrate granules, and carboxysomes. This is possibly an adaptive mechanism to withstand the freezing temperature and high intensity of UV light exhibited in polar regions. Irrespective of the isolates, glycolipid and polysaccharide layer of the heterocyst remained opaque in all the three strains.

Identification of organisms was also confirmed by 16S RNA gene as marker sequence to infer their phylogenetic position. A phylogenetic tree of nearly complete 16S rRNA gene sequence (~1380) was constructed using 37 cyanobacterial sequences by maximum likelihood method (Fig. 3). The clustering pattern of the *Nostoc* strain was found to be based on the occurrence of their habitats. Cluster I (A, B sub clusters) comprised of *Nodularia* strains isolated from various habitats which had been used to characterize the genus *Nodularia* in the earlier studies (Lehtimaki et al. 2000). Cluster II comprised of *Cyanospira* and *Anabaenopsis* strains of alkaline ponds in two subclusters IIA and IIB, respectively. The marine mesophilic strain *Nostoc calcicola* BDU 40302 and *Nostoc calcicola* BDU 180601 appeared in cluster III with the highest statistical support. Clusters IV and V

Fig. 2 Transmission electron micrographs **a, b** *Nostoc punctiforme* BDU ARC 10101, **c, d** *Nostoc calcicola* BDU 40302, **e, f** *Nostoc calcicola* BDU 180601, polyhydroxybutyrate granules (phb), cyanophycean granules (cg), carboxysomes (c), thylakoids (t), sheath (s) taken at the magnification as indicated by the scale bar (1 μm) except, **d** scale bar 500 nm

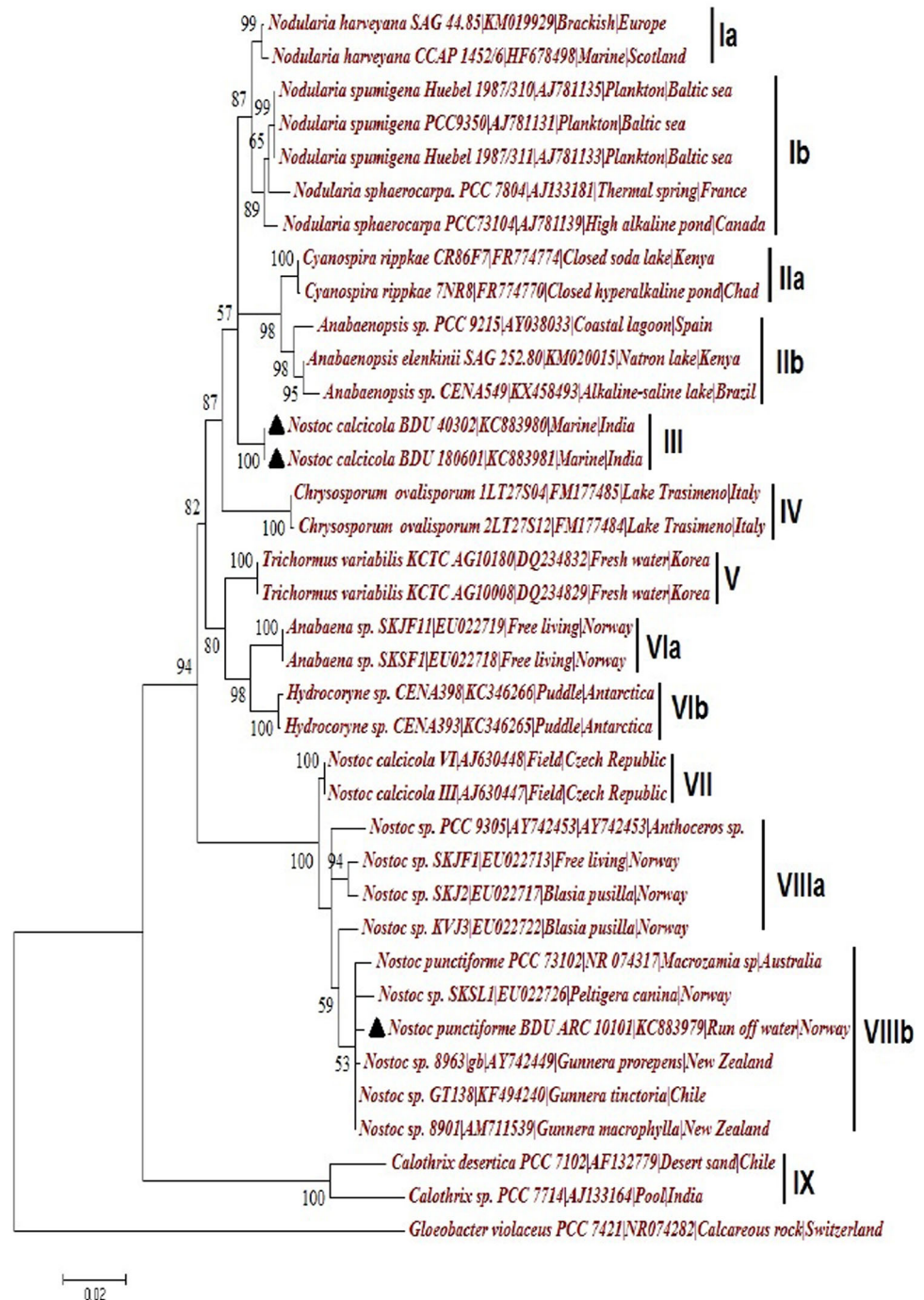


encompassed *Chrysochloris* and *Trichormus* strains of Lake and fresh water origin, respectively. The two sub-clusters VIA and VIB comprised of *Anabaena* and *Hydrocoryne* of Polar Region—Arctic and Antarctic, respectively. Cluster VII encompassed *Nostoc calcicola* III and IV belonged to the agricultural fields of Czech Republic. Cluster VIII consists of most symbiotically associated *Nostoc* strains as two sub clusters (A, B). This was in agreement with the results of earlier studies (Svenning et al. 2005). The psychrophilic *Nostoc punctiforme* BDU ARC 10101 which was isolated from Arctic region clustered with the symbiotically associated *Nostoc* sp. belonging to wide geographical region of Chile, New Zealand, Australia, and Norway. Thus, phylogenetic analysis makes us to accomplish that the psychrophilic cyanobacteria identified as *Nostoc* could be a cyanobiont. It is reported that Arctic cyanobacterial communities are more related to the flora of the continent; whereas the Antarctic communities are secluded (Komarek et al. 2012). *Phormidium autumnale* isolated from Svalbard shared similarity with the strains of Europe and North America (Strunecky et al. 2012). The results of morphology, ultra structure, and 16S rRNA gene analysis make us designate the psychrophilic strain as *Nostoc punctiforme* and mesophilic strains as *Nostoc calcicola*.

Temperature is one of the most significant physical factors that determine the fundamental niche, growth, and life expectancy of a particular species (Cooper et al. 2001). Among the taxonomic groups, green algae and cyanobacteria exhibit their adaptive versatility to both lower and higher temperatures (Staehr and Jensen 2006). The ambient conditions of Arctic are being changed by the impacts of global warming which could affect the nitrogen and carbon cycle in tundra ecosystem (Hayashi et al. 2014). To determine the effect of rising temperature on growth and nitrogen metabolism of psychrophilic *Nostoc* sp., we sought to analyse and compare the short term effects on growth and nitrogen metabolism of psychrophilic and mesophilic *Nostoc* strains of different geographical origins.

The growth of all the three organisms at different temperature regimes such as 4, 15, 25, and 37 °C was measured in terms of dry weight and chlorophyll. The biomass and chlorophyll contents of the psychrophilic strain *Nostoc punctiforme* BDU ARC 10101 though marginally showed enhancement from 4 to 15 °C, got reduced drastically about 50% at 37 °C. The Arctic isolate has been identified as psychrophilic, i.e., they are specially adapted to the lowest optimum temperature to upper limits of 18 °C only. On the other hand, the growth of the mesophilic *Nostoc*

Fig. 3 Phylogenetic estimate on relationship of the studied psychrophilic and mesophilic *Nostoc* strains (indicated with triangle) inferred from 16S rRNA gene sequence (~1380 bp). Number at nodes indicates the bootstrap values (1000 replicates) obtained from the analysis



calcicola BDU 40302 and BDU 180601 with respect to lower temperature showed a decrease in their dry weight and chlorophyll content (Fig. 4a, b) as expected. Earlier studies of temperature response in algae exemplified that the psychrophilic algae *Koliella antarctica*, *Chlorella saccharophila* showed growth up to 15 °C and could not survive at higher temperatures, whereas the mesophilic *Chlorella sorokiniana* exhibited optimal growth at 37 °C (Vona et al. 2004).

Carotenoid, the accessory photosynthetic pigment, plays a vital role in buffering membrane fluidity due to their ability to protect against singlet oxygen-induced damage (Chakraborty et al. 2010). The carotenoid content of the mesophilic strains attained its peak value at 37 °C, whereas in psychrophilic strain, carotenoid content decreased reciprocally to the temperature enhancement (Fig. 4c). Zidarova and Pouneva (2006) have demonstrated a decrease in pigment contents chlorophyll *a*, chlorophyll

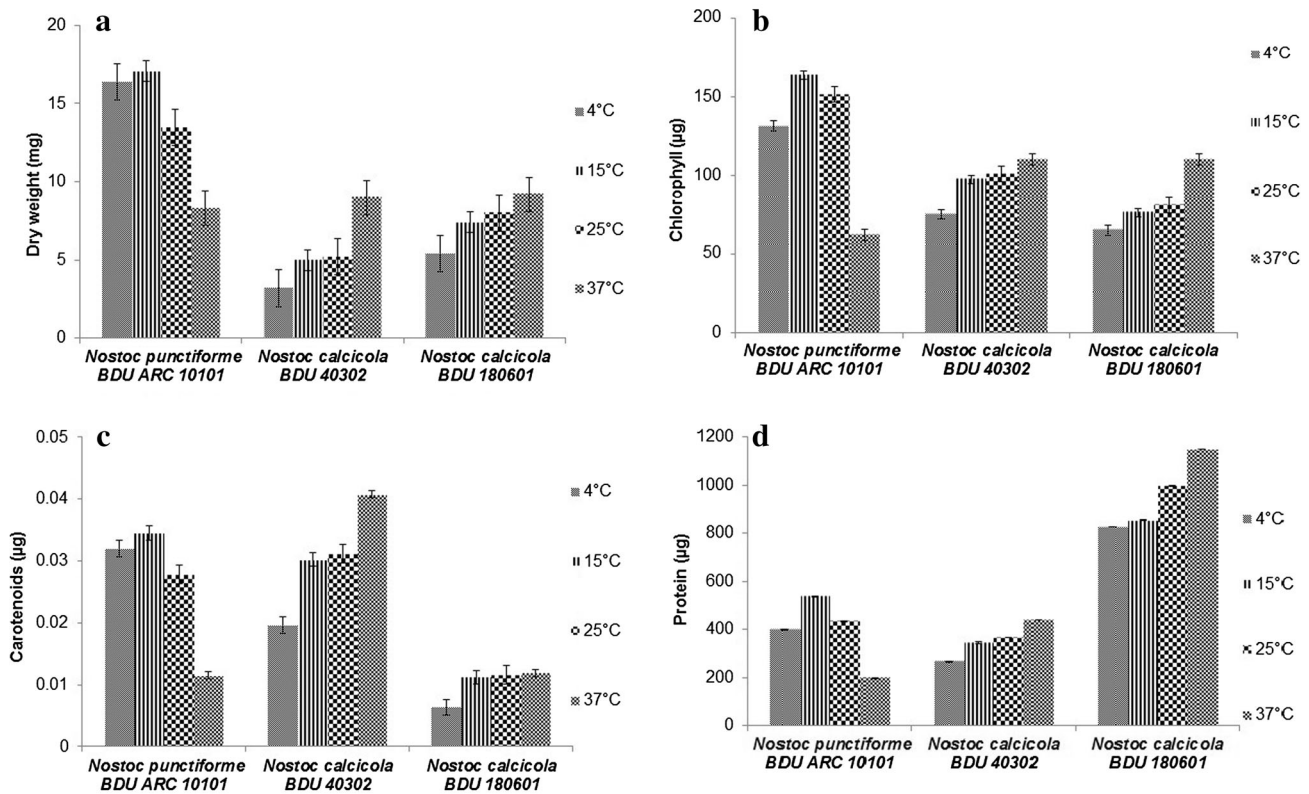


Fig. 4 Growth relationship of psychrophilic and mesophilic *Nostoc* strains at different temperatures **a** dry weight, **b** chlorophyll, **c** carotenoids, and **d** protein

b and β -carotene of the psychrophilic strain, *Chlorocystis minor* at extreme high temperature. In the present study, psychrophilic *Nostoc* strain synthesized a higher amount of carotenoids at a lower temperature compared to higher temperature. In contrast, the carotenoid content of the mesophilic strains got reduced at the lower temperature and increased at the higher temperature. Chintalapati et al. (2004) has demonstrated increased carotenoid synthesis of two psychrophilic bacteria *Sphingobacterium antarcticus* at low temperature indicating the stability of the membrane and supports our finding. Alike pigments, the protein content of the psychrophilic *Nostoc* also was maximum at 15 °C which prominently lessened at 37 °C. The protein content of the *Nostoc calcicola* BDU 40302 and *Nostoc calcicola* BDU 180601 was minimal 266 and 826 μg at 4 °C and maximum 440 and 1149 μg at 37 °C, respectively (Fig. 4d).

Nitrogen fixation represents an important input of nitrogen to arctic ecosystem (Viderova et al. 2011). The nitrogenase activity of mesophilic strains got downregulated at 4 °C showing its sensitivity to lower temperature. At the same time, the psychrophilic strain, an Arctic isolate, was able to fix nitrogen at 4 °C and its activity increased reciprocally to the temperature enhancement (Fig. 5a). Similar effects of increased activity at enhanced

temperature were reported in psychrophilic purple mat (PM) community of Maritime Antarctica comprising N_2 fixing cyanobacteria (Velazquez et al. 2011). The ability of psychrophilic strain to fix nitrogen regardless of the temperature could be due to the fact of tightly entangled trichomes with multi-layered sheath which creates a strict anaerobic environment protecting nitrogenase, the oxygen labile enzyme, thereby enhancing N_2 fixation.

Nitrogen fixation and its assimilation by cyanobacterial cell rely on cellular energy and are a complex interconnected metabolic cycle (Debnath et al. 2012). Glutamine synthetase (GS) is an efficient ammonia scavenger in cyanobacteria (Priya et al. 2011). At lower temperatures of 4 and 15 °C, psychrophilic strain *Nostoc punctiforme* BDU ARC 10101 isolated from nitrogen limited arctic environment exhibited enhanced GS activity to survive in those N limited environment. It has been reported that the organisms survived in nitrogen depleted condition showed controlled expression of genes encoding the nitrate-assimilating system to save energy and also stimulate GS activity (Saha et al. 2003). At higher temperature (37 °C), glutamine synthetase got deactivated in psychrophilic strain (Fig. 5b).

The enzyme nitrate reductase and nitrite reductase synthesize ammonium from nitrate by two sequential

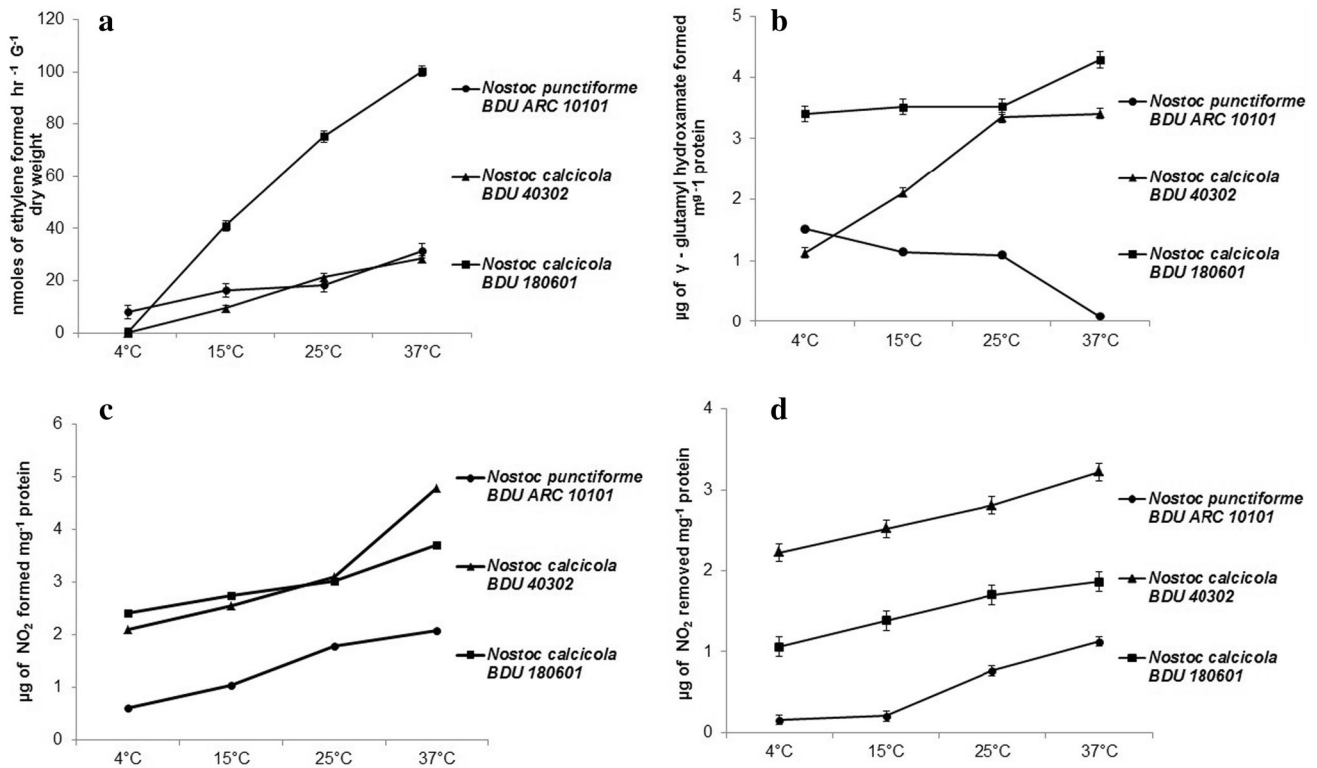


Fig. 5 Effect of temperature on **a** acetylene reduction, **b** glutamine synthetase, **c** nitrate reductase, and **d** nitrite reductase activity of psychrophilic and mesophilic *Nostoc* strains

reactions involving 2-electron and 6-electron reductions and the resulting ammonium ion is incorporated into various intracellular components such as amino acids, protein, RNA and chlorophyll via glutamine synthetase/glutamate synthase (GS–GOGAT) pathway (Flores et al. 2005). In the present study, NR activity reliably got diminished at a lower temperature when compared to the higher temperature. NR enzymes of both psychrophilic or mesophilic strains are stable at higher temperature of 37 °C (Fig. 5c). The NIR activity also exactly followed the same trend in all three *Nostoc* strains regardless of their psychrophilic or mesophilic nature (Fig. 5d). The characteristics of NR from various algae belonging to different thermal habitat showed a wide range of temperature adaptation (Di Martino Rigano et al. 2006). It is reported that the active centre of the enzyme gets structurally altered to shift the enzyme activity optimum to the region of higher temperatures and increased the thermal stability of the NR (Morozkina and Zvyagil'skaya 2007). Decreased enzyme activity at lower temperature may be due to the fact that the affinity for the substrate is greatly decreased at lower temperature (Reay et al. 1999). Di Martino Rigano et al. (2006) reported that NR enzyme from the psychrophilic algae *Koliella antarctica* possesses structural modifications that make it colder adapted, being more catalytically efficient at lower

temperatures, but they are less thermally stable than NR from mesophilic *Chlorella sorokiniana* simultaneously.

The present study related to growth and the nitrogen metabolic enzyme activities clearly demonstrate that the psychrophilic organism has stable and active nitrogen fixing mechanisms at cold temperature congruent with the polar strains. The mesophilic strains have active nitrogen fixation attuned only at its respective temperature. In the case of nitrogen assimilating mechanism, both NR and NIR behaved similarly both in psychrophilic and mesophilic organisms. This might be due to the low availability of combined nitrogen for the organism in the alpine regions which had acclimatized themselves to fix N₂ from the atmosphere at low-temperature efficiently to withstand in this extreme environment.

Conclusion

The morphological analysis encompassing ultrastructure and phylogenetic investigation of the 16S rRNA gene sequence of three isolates of the present study aids in validation of psychrophilic strain as *Nostoc punctiforme* and mesophilic strains as *Nostoc calcicola*. The psychrophilic strain structurally differed from the mesophiles

in having extensive multilayer sheath along with high deposition of cyanophycin, polyhydroxybutyrate granules, and carboxysomes. The findings carried out on the effect of temperature on growth and nitrogen metabolism of psychrophilic and mesophilic *Nostoc* strain corroborates with the survival strategy and thermal adaptive response of the strains in the altered temperature. The significant response observed in metabolic characteristics of nitrogen fixation/assimilation enzymes of *Nostoc* strains at varied temperatures provide valuable insights of physiological contribution and its role in biological nitrogen cycle of various niches.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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