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Short Communication: Effects of temperature on growth, pigment composition and protein content of an Antarctic Cyanobacterium *Nostoc commune*

RANJANA TRIPATHI, UMESH P. DHULDHAJ, SURENDRA SINGH♥

Centre of Advanced Study in Botany, Faculty of Science, Banaras Hindu University, Varanasi 221005, Utar Pradesh, India. Tel: +91-542-6701101; Fax: +91-542-2368174. ♥e-mail: surendrasingh.bhu@gmail.com

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Abstract. *Tripathi R, Dhuldhaj UP, Singh S. 2012. Short Communication: Effects of temperature on growth, pigment composition and protein content of an Antarctic Cyanobacterium* Nostoc commune. *Nusantara Bioscience 4: 134-137*. Effect of temperature variation on biomass accumulation, pigment composition and protein content were studied for the cyanobacterium *Nostoc commune*, isolated from Antarctica. Results confirmed the psychrotrophic behavior (optimum growth temperature 25^0C) of the cyanobacterium. Low temperature increased the duration of lag phase and exponential growth phase. Maximum increase in biomass was recorded on 24^{th} day at 25^0C and on 12^{th} day at 5^0C. The downshift from 25 to 5^0C had almost negligible effect on chl *a* content. Maximal protein content was recorded for cultures growing at 5^0C on 12^{th} day. The carotenoids/chl *a* ratio was maximum (2.48) at 5^0C on 9^{th} day. It remained almost constant for cultures growing at 5 and 35^0C. There was an induction in protein synthesis following downshift in temperature from 25 to 5◦C.

Key words: Cyanobacterium, low-temperature, growth rate, phycobiliproteins, pigments

Abstrak. *Tripathi R, Dhuldhaj UP, Singh S. 2012. Komunikasi singkat: Pengaruh suhu terhadap pertumbuhan, komposisi pigmen dan kandungan protein cyanobacterium dari Antartika* Nostoc commune. *Bioscience Nusantara 4: 134-137*. Pengaruh variasi suhu terhadap akumulasi biomassa, komposisi pigmen dan kandungan protein dipelajari pada cyanobacterium *Nostoc komune*, yang diisolasi dari Antartika. Hasil penelitian menegaskan perilaku psikrotrofik (suhu pertumbuhan optimum 25◦C) dari cyanobacterium tersebut. Suhu rendah meningkatkan durasi fase lambat dan fase pertumbuhan eksponensial. Peningkatan biomassa maksimal tercatat hari ke-24 pada 25◦C dan hari ke-12 pada 5◦C. Penurunan suhu dari 25 ke 5◦C hampir tidak berpengaruh pada kandungan chl a. Kandungan protein tertinggi tercatat pada kultur yang tumbuh pada 5◦C, hari ke-12. Rasio karotenoid/chl *a* tertinggi (2,48) terjadi pada 5◦C, hari ke-9. Hal ini hampir selalu konstan untuk kultur yang tumbuh pada 5-35◦C. Terdapat induksi dalam sintesis protein mengikuti penurunan suhu dari 25 ke 5◦C.

Kata kunci: cyanobacterium, suhu rendah, tingkat pertumbuhan, phycobiliproteins, pigmen

Cold-stress is often lethal to living organisms. For growth to occur in low temperature environments, cellular components such as membranes, proteins and nucleic acids have to adapt to the cold (Cavicchioli et al. 2002). Microbial diversity of the Antarctica is composed of either psychrophilic (optimum growth temperature $<15^0$C) or psychrotrophic (optimum growth temperature $>15^0$C) (Morita 1975; Veerapaneni 2009). Psychrotrophs constitute the bulk of continental Antarctica microflora. Adaptive responses of the Antarctic microbes growing in the permanently cold environments, especially at $0\text{-}4^0$C are little studied (Chattopadhyay 2006). Majority of Antarctic cyanobacteria grow in a wide range of temperature (Nadeau and Castenholz 2000; Nadeau et al. 2001). The temperature-growth response suggests their close relationship with moderate regions of the Antarctic (Seaberg 1981).

Nostoc commune (Nostocales) was collected from Schirmacher Oasis, Antarctica by Dr. Suresh Chandra Singh, a member of the Seventeenth Indian Expedition to Antarctic (Pandey and Upreti 2000). The cyanobacterium was isolated, purified using standard microbiological techniques, and is maintained in nitrogen free BG-11-medium in a culture room set at 25 ± 1^0C, illuminated with day-light fluorescent tubes having the photon fluence rate of 35μE m^{-2} s^{-1} at the surface of vessels. Here, we studied the biomass accumulation (in terms of fresh weight and dry weight ml^{-1} volume of liquid culture harvested at 3 day intervals) and pigment composition of *N. commune*, an Antarctic cyanobacterial to different temperatures (i.e., 5, 15, 25, 35^0C).

Growth of *N. commune* was estimated in terms of chlorophyll *a* (chl *a*) content and expressed in terms of specific growth rate computed as per the method of Myers and Kratz (1955). Growth rate (μ) of *N. commune* at each temperature was estimated as changes in biomass over time from the log-linear portion of the curve using non linear curve fit method with Boltzmann constant. Chl *a* was

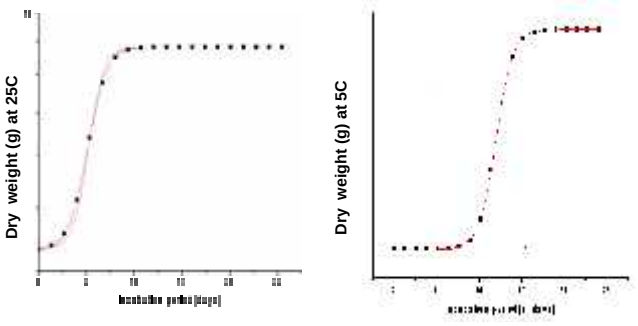
quantified using the formula of Talling and Driver (1963), with 12.7 as extinction coefficient for chl *a* at 663 nm. Carotenoids were estimated according to Myers and Kratz (1955). Phycobilliproteins (PBPs) (both qualitatively and quantitatively) were analysed by recording the absorption spectra and absorbance at various wavelengths in a UV-VIS spectrophotometer (Varian, Cary100-Bio, USA) with a 1 cm light path. Fluorescence excitation and emission spectra were recorded in a fluorescence spectrophotometer (Hitachi F-2500, Japan). The amounts of different PBPs namely, phycocyanin (PC, A_{max} 565 nm), allophycocynain (APC, A_{max} 620 nm) and phycoerythrin (PE, A_{max} 650 nm) were determined according to the equations given by Tandeau and Houmard (1993). Protein was estimated following the method of Lowry et al. (1951) using lysozyme as the standard.

The specific growth rate (K) and $\log_{10}$ specific growth rate ($\log_{10}$ K) of *N. commune* growing at different temperatures (5 to 35^{0}C) are shown in Table 1. The optimum temperature (T_{opt}) for growth of *N. commune* ranged in between 15 and 25°C with less difference in μ_{max} values. The specific growth rate of *N. commune* was maximal at 25^{0}C. Maximum growth rate was 1.9590 day^{-1} at 15^{0}C. The Q_{10} value ranged from 2-3. Results suggest psychrotrophic behavior of *N. commune*.

Table 1. Effect of temperature on growth characteristics of *N. commune.*

Temperature (^{0}C)	μ	Log K_{10}
5	0.48	0.5972
15	1.0072	1.9590
25	1.48	1.2900
35	0.48	0.4800

Curve fitting results suggest that *N. commune* grew exponentially from 3 to 9 day at 25^{0}C, and 9 to 15 day at 5^{0}C (Figure 1). After a downshift in temperature from 25 to 5^{0}C, exponential phase of the cyanobacterium moved from 3 to 9 day. Duration of lag phase also increased following the downshift. The stationary growth phase started from 15th day for cultures growing at 5^{0}C, and continued with increase in incubation period. This indicates the adaptability of *N. commune* to low temperature (5^{0}C), and its preference for 25^{0}C. Maximum increase in biomass was recorded at 24th day at 25^{0}C and on 12th day at 5^{0}C (Figure 2).



A B

Figure 1. Best fit curve of *Nostoc commune.* A. at 25^{0}C, B. at 5^{0}C.

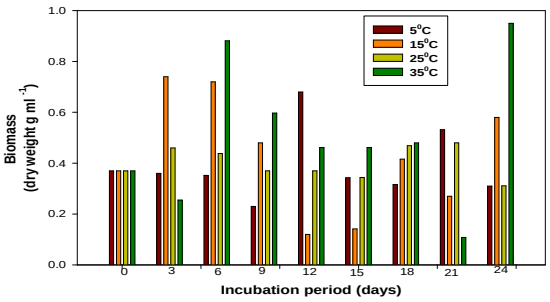


Figure 2. Biomass accumulation by *N. commune* at different temperatures (5-35^{0}C).

Maximum amount of chl *a* was recorded on 27th day for cultures growing at 25^{0}C. The downshift from 25 to 5^{0}C had almost negligible effect on chl *a* content. The chl *a* content increased from 0.49 to 0.703 µg mL^{-1} at 35^{0}C during first 6 days of incubation and remained constant thereafter (Figure 3). Maximal protein content was recorded for cultures growing at 5^{0}C on 12th day. Protein synthesis was exponential during 9 to 15 days (Figure 4).

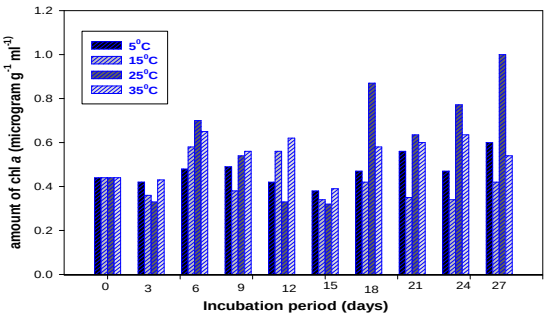


Figure 3. Effect of temperature (5-35^{0}C) on chl *a* content of *N. commune.*

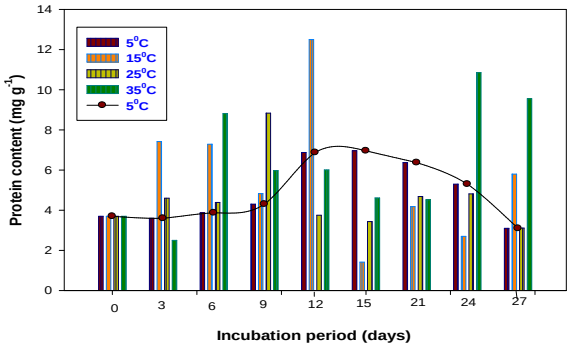


Figure 4. Effect of temperature (5-35^{0}C) on protein content of *N. commune.*

PC/chl *a* ratio was highest on 21st day at 35^{0}C. At 5^{0}C, the ratio of PC/chl *a* increased upto 6th day, decreased thereafter. A minimum increase in PC/chl *a* ratio (0.0148 and 0.0126) was recorded on day 15th (also 18 day) at 5^{0}C. The maximum increase in PC/chl *a* ratio (0.1876) was recorded on 21 day at 25^{0}C (Figure 5A). PE/chl *a* ratio was maximum on 21st day for cultures growing at 35^{0}C. However, cultures growing at 5 and 15^{0}C exhibited minimum PE/chl *a* ratio on 15th and 18th day, respectively. The ratio increased upto 12th day at 5^{0}C and declined thereafter (Figure 5B). APC/chl *a* ratio was found to be highest on 24th day at 15^{0}C. Its value for cultures growing at 5^{0}C increased from 12th day (Figure 5C).

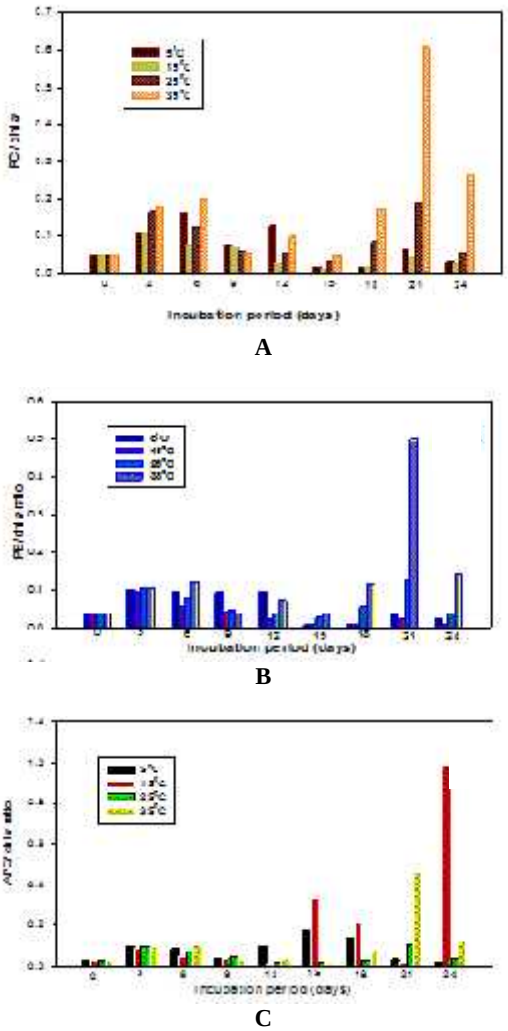


Figure 5. Effect of temperature on *N. commune*: A. PC/chl *a* ratio, B. PE/chl *a* ratio, C. APC/chl *a* ratio.

The carotenoids/chl *a* ratio was maximum (2.48) on 9th day at 5^{0}C. It remained almost constant for cultures growing at 5 and 35^{0}C (Figure 6). Protein/chl *a* ratio increased upto 24th day at 5^{0}C. There was an induction in protein synthesis following downshift in temperature from 25 to 5^{0}C (Figure 7).

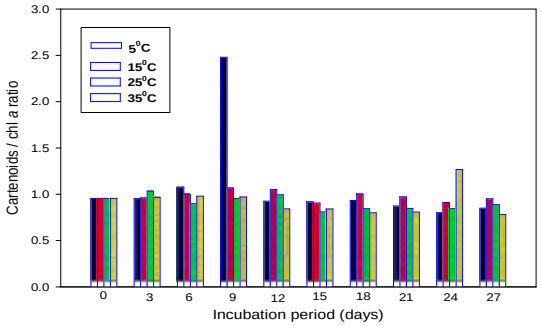


Figure 6. Effect of temperatures on carotenoids/chl *a* ratio of *N. commune*.

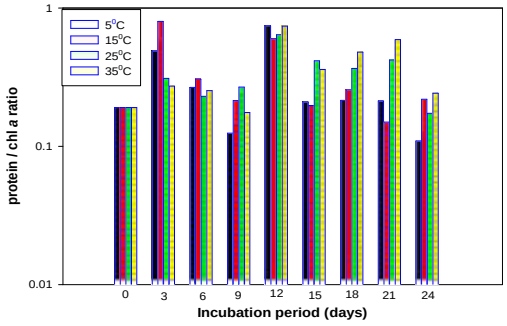


Figure 7. Effect of different temperature on protein/chl *a* ratio of *N. commune*.

The growth and survival of cyanobacteria inhabiting Antarctic environments are less understood. *N. commune* grew within a range of 10-30^{0}C, suggesting eurythermal (broad tolerance range) nature of the cyanobacterium. Reduced growth at low temperature (e.g. ≤ 5^{0}C) and responsiveness to the temperature changes suggest that in Antarctica cyanobacteria could accumulate biomass during the brief periods of summer.

The increase in carotenoids/chl *a* ratio at low temperature could help the cyanobacterium from protection against photooxidation, resulting at low temperature (Young 1991; Chalifour and Juneau 2011). The consistent decrease in phycobilliproteins/chl *a* ratio with increasing temperature suggests that all pigment ratios (including carotenoids/chl *a*) are controlled primarily by cellular chl *a* content (biosynthesis).

The algal communities in the polar environments are exposed to continuous high irradiance during the summer. This, in combination with the low temperature, it increases the chances of photoinhibition (Bascuñán-Godoy et al. 2012). It was reported that carbon fixation limits growth and photosynthesis at low temperature, and algae tend to direct resources away from the synthesis of light-harvesting components at low temperature (Alves et al. 2002)

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