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Research

# Nitrate Reductase Activity of Thermophilic Cyanobacteria *Mastigocladus* sp.

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**Abstract:** The thermophilic cyanobacteria have adapted themselves to a wide array of ecological niches such as hot springs, volcanoes, etc. In cyanobacteria lipids, nucleic acids, and proteins are susceptible to heat and therefore there is no single factor that enables all thermophiles to grow at extreme temperatures. The lipid membranes of thermophiles have saturated and straight chains of fatty acids to provide the right degree of fluidity needed for membrane function. Some species contain Para crystalline surface layers which function as external protective barriers. Thermophilic cyanobacteria also have histone-like protein that protects DNA and have reverse gyrase which is responsible for positive supercoiling in DNA. Heat shock protein chaperons are also present which are likely to play a significant role in stabilizing and refolding proteins as they begin to denature. The study has unraveled a lot of significant data and opened many new avenues of research in the industrial use of extremophiles, which answer the fundamental questions of the existence of extremophilic organisms on Earth and their use in various biotechnological processes.

**Keywords:** Cyanobacteria, Nitrate reductase activity, Thermophiles

## Introduction

Thermophilic cyanobacteria are the most studied extreme group in the world. Thermophilic microorganisms are found in the thermal waters of each continent except Antarctica. The inhabitants of these thermal environments are called thermophiles ("thermo" for heat, "phile" for lover—survive and thrive). 'A thermophile is an organism capable of living at a temperature at 'or' near the maximum for the taxonomic group of which it is a part.' (Brock 1986). These thermophiles are found in hot springs and hydrothermal vents of the world.

Geothermal springs of temperature between 45-100°C are almost exclusively inhabited by thermophilic prokaryotic microorganisms, photosynthetic flexi bacteria, and certain non-photosynthetic autotrophic and heterotrophic bacteria. (Pentecost 2003, Banerjee and Castenholz 2009, Norris and Castenholz 2007). The hot source of water contains most of the essential nutrients and permits a steady growth of cyanobacteria throughout the year. As the effluent moves away from the source of the spring the water cools thus setting up a temperature gradient (Banerjee et al., 2001, Banerjee and Verma 2008). Downstream as the temperature gradually decreases from 73°C to about 53°C, populations of unicellular and filamentous heterocystous cyanobacteria, form deep green to reddish brown mats (Bhattacharya 2001). Thermophilic cyanobacteria have adapted themselves to a wide array of ecological niches such as hot springs, volcanoes, etc. (Madigan and Brock 1977).

These algae withstand 'or' tolerate a very high temperature. (Castenholz 1977). Thermophilic cyanobacteria have become an experimental system for basic and applied research due to their unique properties (Whitton et al. 1990, Tomitani et al. 1999)

Natural geothermal areas are widely distributed around the globe, but they are primarily associated with tectonically active zones at which the movements of the Earth's crust occur (Castenholz 1969, 1977). Due to this localization of geothermal heat sources, hot springs are generally restricted to a few concentrated areas. The most important biotopes are terrestrial geothermal fields, with alkaline freshwater hot springs and solfatara, and marine environments with coastal, shallow, and deep hydrothermal systems. Hot environments display a complete range of pH, from acid to alkaline, depending on temperature, water availability, and gas and ion concentration (Kristjansson and Hreggvidsson 1995, Haverkamp et al. 2008).

Thermophilic cyanobacterium *Mastigocladus* is dominant in the thermal hot springs of every continent. Description of this cyanobacterium was first given by Ferdinand Cohn (1862) from Karlsbad Hot Spring (now Karlovy Vary in the Czech Republic). He pointed out the evolutionary and biogeochemical significance of organisms living in the hot spring. The organism forms tough mats and the morphology of this cyanobacterium is very simple. The cells of the main filament are long cylinder and barrel in shape with narrow branches at one side with an intercalary heterocyst. The cells of side branches are long cylinders. Hormogonia are absent in this organism (Jha et al. 1986, Bhattacharya 2001, Khumanthem et al. 2007).

Among all the thermophilic microorganisms *Mastigocladus* has a unique ability to adapt entire range of temperatures and perform oxygenic photosynthesis in addition to the ability to fix atmospheric nitrogen, especially at elevated temperatures (Fogg 1951, Stewart 1970, Miller et al. 2006**,** Khumanthem et al. 2007, Miller et al. 2009). In India, *Mastigocladus* was found in the thermal hot spring of Barkeshwar (West Bengal), Rajgir (Bihar) and it is also found in the thermal effluents of the Tarapur nuclear plant, near Maharashtra (Bhattacharya 2001).

Cyanobacteria are fundamentally important colonists of hot springs and hydrothermal vents. Their role in these extreme ecosystems is a result of their remarkable resistance to extreme temperature and high salinity whilst being capable of photosynthesis in adverse situations. Their production of compatible solutes makes them tolerate osmotic stresses resulting from desiccation, hot temperatures, and extreme salinity.

Although such initial research on thermophilic *Mastigocladus* there is a paucity of knowledge regarding the behavior of this organism when isolated from their natural environment to lab conditions. The study of this organism, therefore, is not only important because it is biologically and scientifically very interesting but also because there is a great lacuna of knowledge regarding the physiology of nitrate metabolism in this organism which is essential for its growth and survival in the niche it belongs. Almost nothing is known about the mechanism adopted by these organisms to perform nitrate reduction metabolism under extreme conditions. Keeping this in mind, this study is a sincere attempt to study thermophilic cyanobacteria, heterocystous *Mastigocladus* from the hot springs of Yellowstone National Park its nitrate metabolism.

## Materials and Methods

### Collection and Identification of the Microbes

The culture of *Mastigocladus* has been isolated from the thermal hot springs of Yellowstone National Park, the organism was available in the Centre for Ecology and Evolutionary Biology, University of Oregon, USA. *Mastigocladus* was cultured, purified, identified, and made axenic by standard microbiological procedure by Professor (Dr.) Meenakshi Banerjee Head, Department of Biosciences, Barkatullah University Bhopal during her visit to that center in the USA in 2007, and brought to the laboratory of Algal Biotechnology for further work. Identification of the strains was made according to the standard literature like Desikacharya (1959), the latest edition of Bergey's Manual (2001). Now isolated cultures were a part of the culture collection of the Laboratory of Algal Biotechnology, Department of Bioscience, Barkatullah University, Bhopal (Madhya Pradesh).

**Cultural Media and Culture Conditions**

Out of several media tested *Mastigocladus* showed the best growth in Fogg's ($N^-$) medium where $A_5$ and FeEDTA were autoclaved separately.

**Culture Vessels**

The glassware used was either Borosil or Qualigens made. Cultures were maintained in culture tubes (15x1.5 cm) each containing 15ml medium in Erlenmeyer flasks of 100 ml or 250 ml capacity containing 20 ml or 100 ml medium respectively.

**Sterilization of the Medium and Glassware**

Culture medium and culture vessels were sterilized by wet-heat sterilization method in an autoclave at 15 lb/inch$^2$ pressure and a temperature of 121°C for 15-20 minutes.

**Incubation and Maintenance of Enrichment Culture**

The culture was incubated in an air-conditioned culture room and illuminated by three 40 W fluorescent tubes at 50 cm for 16 hours daily unless otherwise stated in the case of light-intensity experiments. The culture was shaken in an orbital shaking incubator as well as by hand. The cultures were sub-cultured at regular intervals and experiments were conducted on exponentially growing cultures only.

**Nitrate Reductase Activity**

The estimation of *in vivo* nitrate reductase activity was done by the following method of Camm and Stein (1974) as slightly modified by Kumar and Kumar.

**Results**

Figure 1 shows the graph of nitrate reductase activity of *Mastigocladus*. In *Mastigocladus* nitrate reductase activity was highest at 96 h (0.74 µg $NO_2$ µg Chl *a*/ ml$^{-1}$) after which there was a decline in the activity. The effect of temperatures has been studied on the strain. In *Mastigocladus* with increasing temperature an increase in nitrate reductase activity was observed, the activity was highest at 55±2ºC (0.84 µg $NO_2$ / µg Chl *a* ml$^{-1}$) at 96 h (4.3b), and the fold increase was found to be 1.13 over the control (25±2ºC) at 96 h.

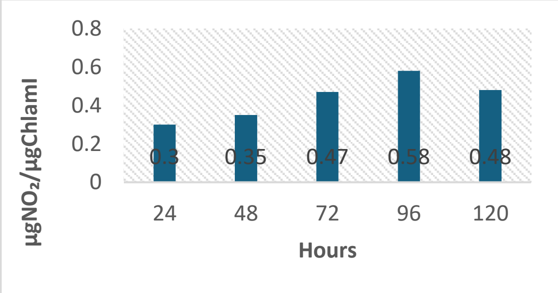


**Fig. 1.** Nitrate reductase of *Mastigocladus* (Fogg's $N^-$) under laboratory conditions. (Temperature 25ºC± 2ºC, Light Intensity 2500±200 Lux, pH- 8.2, Results mean ± SD n=3).

**Discussion**

It is observed in a present study that nitrate reductase activity high in *Mastigocladus.* In *Mastigocladus* nitrate reductase is the main nitrogen assimilating enzyme like nitrogenase activity. If nitrate is available, then *Mastigocladus* can choose between the energy-expensive nitrogenase and nitrate reductase. Studies on nitrate reductase activity showed that if provided with external sources of nitrogen, the extremophiles could metabolize it. Under extreme conditions, the morphology of the cyanobacteria changes remarkably, and this could cause enormous effects, changing the whole metabolism of the organism, especially at the biochemical level. Under elevated temperature conditions as studied and like that found in nature the nitrate reductase activity increased in *Mastigocladus* (Fig. 1). This suggests that it can be the adaptation of the organisms for elevated temperature, at elevated temperature the surface area of the cell increases which provides a far greater area to capture light energy and hence increased photosynthesis resulting in increased reductant energy supply for the enzyme activities (Bhattacharya 2001). Nitrate assimilation depends upon the $CO_2$ fixation. The high nitrate reductase activity in the thermophile at normal temperature (25ºC±2ºC) as well as at elevated temperature showed the flexibility of the enzyme, in the organism.

The main results so far point to the importance of the thermophile *Mastigocladus* which is thermostable even when isolated under culture conditions and maintained in culture room. Such organisms therefore have tremendous applications in research requiring thermostability. This study paves the path for the future utilization of *Mastigocladus* in biofertilizers in regions of remarkably elevated temperature, biological research about the use of thermostable enzymes, and industrial and biotechnological applications.

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