

Role of antioxidative enzymes in toxic bloom forming Cyanobacteria

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Abstract: The antioxidative enzymes catalase and ascorbate peroxidase in toxic bloom forming cyanobacteria play a pioneer role in scavenging free radicals, which are generated as an outcome of photosynthesis and respiration. Ten Bloom forming toxic cyanobacterial strains *Synechococcus elongates*, *Synechocystis aquatalis*, *Merismopedia glauca*, *Microcystis aeruginosa*, *M. aeruginosa* (O), *Arthrospira* (=Spirulina) *platensis*, *Nostoc paludosum*, *Anabaena iyengarii*, *A. variabilis* and *Cylindrospermum muscicola* were isolated from natural blooms and purified. The results depict the significant activity of antioxidative enzymes. In *A. platensis* the activity of catalase (28.7 ± 2.3 mM/min/ μ g protein), and Ascorbate peroxidase (1.91 ± 0.12 mM/min/ μ g protein) enzyme was maximum. It was also observed that the activity of ascorbate peroxidase was very less compared to activity of catalase. The growth analysis and pigment profile were also studied. Growth measurements revealed that cells attain maximum growth nearly after 15-20 days of inoculation except *S. aquatalis*, *A. platensis* and *N. paludosum maximum*, where optimum growth was achieved after 25th day. Chlorophyll-a content of *A. platensis* showed maximum concentration (14.47 ± 1.17 mg/L) and minimum concentration in *S. elongates* (2.30 ± 0.138 mg/L). The concentration of phycobiliproteins was found maximum in *A. platensis* (2.09 mg/ml). The detection of antioxidative potential of cyanobacterial strains will help to broaden the knowledge about their survival and can pave path for their utilization in biotechnology.

Keywords: Antioxidative enzymes, Ascorbate peroxidase, Catalase, Cyanobacteria, Toxic Blooms

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes. They possess very simple cellular structure. The photosynthetic apparatus is represented by isolated and freely lying photosynthetic lamellae. Chlorophyll *a* and several accessory pigments (phycoerythrin and phycocyanin) are embedded in these photosynthetic lamellae. The major photosynthetic pigment in cyanobacteria is Chlorophyll – *a*. The outer surface of thalokoids of cyanobacteria contains phycobilisomes, which are light harvesting clusters of phycobiliproteins. The phycobilisome components (phycobilin) are responsible for the blue-green pigmentation of most cyanobacteria. They absorb light in the visible region and transfer the excitation energy to Chl-*a* molecule. Phycobiliproteins are major photosynthetic accessory pigments of cyanobacteria (Minu *et al.*, 2011).

The toxins secreted by cyanobacteria can be hepatotoxic, neurotoxic, cytotoxic or dermatotoxic in their mode of action. The exploration of antioxidative potential of these bloom forming toxic cyanobacteria is of immense importance. Reactive oxygen species are generated because of aerobic respiration and oxygenic photosynthesis. The active oxygen species are capable of damaging membranes, proteins, pigments and genetic

material, disrupting the metabolism of the organism (Shaaltiel and Gressel, 1986, Scandalios, 1993). Cyanobacteria and plants have developed a number of antioxidative systems for management of free radicals. Early reports indicated the presence of two major hydrogen peroxide scavenging activities in cyanobacteria, catalase and ascorbate peroxidase (Tel-Or *et al.*, 1985, 1986).

Catalase activity has been found in all cyanobacteria species (Miyake *et al.*, 1991). Catalase (EC 1.11.1.6) is an extraordinary enzyme as the reaction it catalyses is crucial to life. It catalyses conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent to water and molecular oxygen (Sichak and Dounce, 1986; Halliwell and Gutteridge, 1999). These enzymes also use hydrogen peroxide to oxidize toxins including phenols, formic acid, formaldehyde, ethanol, methanol or elemental mercury. Catalases have four heme containing subunits, which are responsible for carrying out catalase's activity (Fita and Rossmann, 1985; Darr and Fridovich, 1986).

Ascorbate peroxidase (APX) is a hydrogen-scavenging enzyme. It is a member of the heme peroxide family of enzymes, which are found in most life forms, higher plants, yeasts, algae and some cyanobacteria. They have many biological functions including antibacterial action,

hormone synthesis and hydrogen peroxide removal. Ascorbate peroxidase (EC 1.11.1.11) exists as isozymes and plays important role in the metabolism of hydrogen peroxide. A lot of work on the purification, molecular cloning and physiological functions of APX isoenzymes has been reported (Nakano and Asada, 1981, Mittler and Zilinkas, 1994). This utilizes ascorbate as its electron donor to reduce hydrogen peroxide to water with the concomitant generation of monodehydroascorbate (MDAsA), a univalent oxidant of ascorbate. APX in combination with the effective AsA-GSH cycle functions to prevent the accumulation of toxic levels of hydrogen peroxide in photosynthetic organisms (Asada, 1992, 1997). The aim of the present study was to explore the antioxidative potential of catalase and ascorbate peroxidase in toxic bloom forming cyanobacteria.

MATERIALS AND METHODS

Ten cyanobacterial strains belonging to genera *Synechococcus elongates*, *Synechocystis aquatilis*, *Merismopedia glauca*, *Microcystis aeruginosa*, *M. aeruginosa* (O), *Arthrospira* (= *Spirulina*) *platensis*, *Nostoc paludosum*, *Anabaena iyengarii*, *A. variabilis* and *Cylindrospermum muscicola* which are the major bloom forming forms in Northern India were taken for the study and were isolated from natural blooms (Rippka *et al.*, 1979, Rippka, 1988). The strains were purified and enriched in the laboratory in BG-11 medium (Stanier *et al.*, 1971).

Growth rate was measured by recording the absorbance at 660 nm from the day of inoculation, after every five days until 40th Day (Ohtake *et al.*, 1989). Protein was extracted by suspending the cell pellet in 1 ml 100 mM phosphate buffer (pH 7.5) containing Lysozyme (0.5 mg/ml), EDTA (5 mM), PMSF (1 mM), PVP (1%) and Triton (0.1%). The mixture was incubated on ice for 30 minutes followed by centrifugation at 10,000 rpm for 30 minutes at 4° C and supernatant stored at -20° C.

Protein concentration was determined using Bovine serum albumin as a standard (Bradford, 1976). The quantification of Chlorophyll-a was done in methanolic extracts by absorbance at 665 nm and 652 nm (Porra *et al.*, 1989). Concentration of C-phycoerythrin (PE), C-phycoerythrin (PE) and Allophycocyanin (APC) was calculated using the equations derived from extinction coefficients of purified phycobiliproteins (Bennett and Bogorad, 1971).

For extraction and determination of Catalase (EC 1.11.1.6), fresh cyanobacterial culture (10 ml) was taken and centrifuged at 8,000 rpm for 15 minutes at 4° C and the pellet was kept on ice. The pellet was homogenized with 100 mM phosphate buffer (pH 7.0) containing 0.5mg/ml Lysozyme, 5mM EDTA, 1mM PMSF, 1% PVP and 0.1% Triton. Incubation was done on ice for 30 minutes

followed by centrifugation at 10,000 rpm for 30 minutes at 4° C. Catalase activity was determined by monitoring the consumption of hydrogen peroxide at 240 nm for 5 minutes (Rao *et al.*, 1996). For measurement of APX activity, 10 ml of fresh culture was taken and centrifuged at 8,000 rpm for 15 minutes at 4° C and the pellet was homogenized in 100 mM potassium phosphate buffer (pH 7.5) containing 5 mM EDTA, 1 mM PMSF, 1% PVP 0.1% Triton and 5 mM ascorbate. Incubation was done on ice for 30 minutes followed by centrifugation at 10,000 rpm for 30 minutes at 4° C. APX activity was determined by following the decrease in absorbance at 290 nm for 3 min (Nakano and Asada, 1981).

RESULTS

Growth of cyanobacterial cultures depends on the conditions like light, temperature, nutrients etc. In this, study growth measurements revealed that cells attain maximum growth nearly after 15-20 days of inoculation. But in *S. aquatilis*, *A. platensis* and *N. paludosum* maximum growth was achieved after 25th day. After maximum growth, the cells begin to die due to excess of metabolites in the medium (Fig. 1).

The protein content of *A. iyengarii* ($12.78 \pm 0.97 \mu\text{g}/\mu\text{l}$) was found to be maximum followed by *A. variabilis* ($6.41 \pm 0.68 \mu\text{g}/\mu\text{l}$), *N. paludosum* ($5.28 \pm 0.45 \mu\text{g}/\mu\text{l}$), *C.* ($3.16 \pm 0.24 \mu\text{g}/\mu\text{l}$), *S. elongates* ($2.5 \pm 0.15 \mu\text{g}/\mu\text{l}$), *M. glauca* ($2.5 \pm 0.19 \mu\text{g}/\mu\text{l}$), *M. aeruginosa* ($2.3 \pm 0.11 \mu\text{g}/\mu\text{l}$), *M. aeruginosa* (O) ($1.3 \pm 0.08 \mu\text{g}/\mu\text{l}$), *A. platensis* ($2.2 \pm 0.26 \mu\text{g}/\mu\text{l}$) and *S. aquatilis* ($1.6 \pm 0.13 \mu\text{g}/\mu\text{l}$) (Fig. 2).

The quantification of Chlorophyll-a was done in methanolic extracts by recording absorbance at 665 and 652 nm. In terms of chlorophyll-a content *A. (=Spirulina) platensis* showed maximum concentration $14.47 \pm 1.17 \text{ mg/L}$ followed by *S. aquatilis* ($12.82 \pm 1.54 \text{ mg/L}$), *M. glauca* ($12.31 \pm 1.10 \text{ mg/L}$), *A. iyengarii* ($7.50 \pm 0.67 \text{ mg/L}$), *N. paludosum* ($7.16 \pm 0.98 \text{ mg/L}$), *A. variabilis* ($6.28 \pm 0.71 \text{ mg/L}$), *C. muscicola* ($5.43 \pm 0.42 \text{ mg/L}$), *M. aeruginosa* ($6.2 \pm 0.65 \text{ mg/L}$), *M. aeruginosa* (O) ($5.48 \pm 0.59 \text{ mg/L}$), and *S. elongates* ($2.30 \pm 0.138 \text{ mg/L}$) (Fig. 3).

The concentration of phycobiliproteins was found maximum in *A. (=Spirulina) platensis* (2.09 mg/ml) followed by *S. aquatilis* (1.84 mg/ml), *M. glauca* (0.904 mg/ml), *A. iyengarii* (0.738 mg/ml), *N. paludosum* (0.714 mg/ml), *A. variabilis* (0.699 mg/ml), *C. muscicola* (0.641 mg/ml), *S. elongates* (0.588 mg/ml), *M. aeruginosa* (0.517 mg/ml), and *M. aeruginosa* (O) (0.498 mg/ml) (Fig. 4).

The activity of antioxidative enzyme- catalase was found in all ten cultures studied. The enzymatic activity of catalase in *S. elongates* ($3.67 \pm 0.38 \text{ mM/min}/\mu\text{g protein}$), *S. aquatilis* ($15.18 \pm 1.5 \text{ mM/min}/\mu\text{g protein}$), *M. glauca* ($8.17 \pm 0.89 \text{ mM/min}/\mu\text{g protein}$), *M. aeruginosa* ($4.12 \pm 0.59 \text{ mM/min}/\mu\text{g protein}$), *M. aeruginosa* (O) ($3.76 \pm 0.44 \text{ mM/min}/\mu\text{g protein}$), *A. platensis* ($28.7 \pm 2.3 \text{ mM/min}/\mu\text{g}$

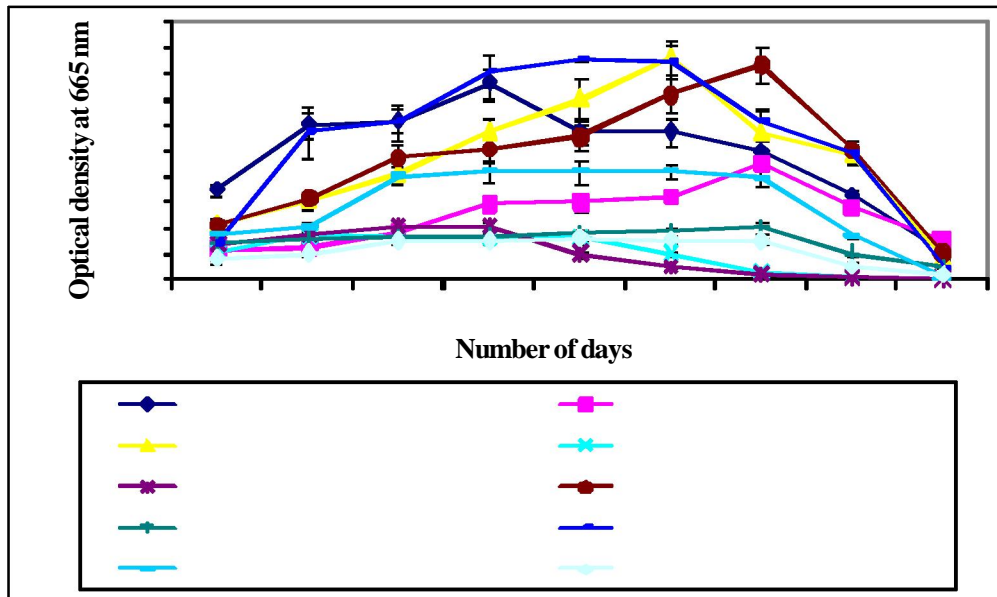


Fig.1. Growth curve of cyanobacteria.

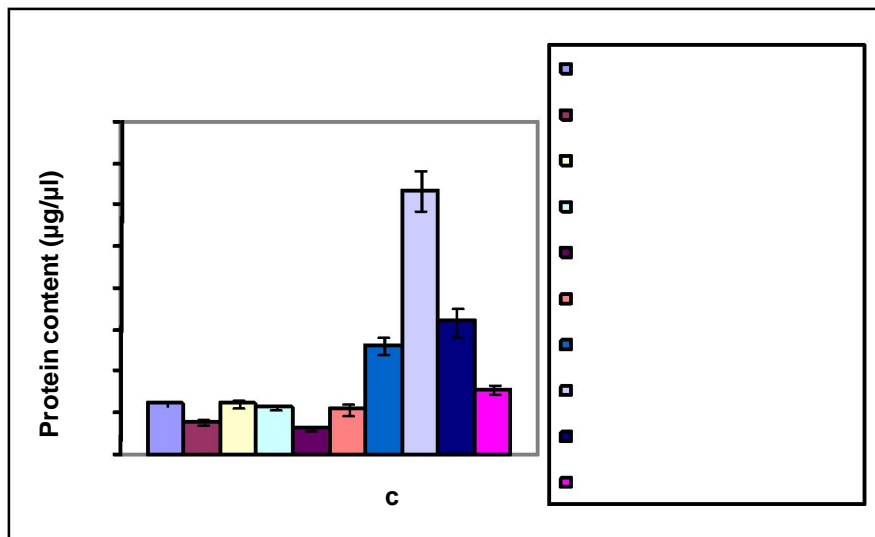


Fig.2. Protein concentration of cyanobacterial cultures.

protein), *N. paludosum* (2.84 ± 0.24 mM/min/ μg protein), *A. iyengarii* (4.89 ± 0.23 mM/min/ μg protein) *A. variabilis* (4.36 ± 0.55 mM/min/ μg protein), *C.muscicola* (4.23 ± 0.43 mM/min/ μg protein) was observed (Fig.5).

Activity of ascorbate peroxidase: The activity of enzyme ascorbate peroxidase was lesser compared to the catalase activity. The ascorbate activity in *S. elongates* (0.161 ± 0.02 mM/min/ μg protein), *S. aquatalis* (0.9 ± 0.06 mM/min/ μg protein), *M. glauca* (0.913 ± 0.035 mM/min/ μg protein), *M. aeruginosa* (1.35 ± 0.22 mM/min/ μg protein), *M. aeruginosa* (O) (0.440 ± 0.03 mM/min/ μg protein), *A. platensis* (1.91 ± 0.12 mM/min/ μg protein), *N. paludosum* (0.58 ± 0.01 mM/min/ μg protein), *A. iyengarii* (0.97 ± 0.027 mM/min/ μg protein) *A. variabilis* (0.57 ± 0.03 mM/min/ μg protein), *C. muscicola* (0.388 ± 0.05 mM/min/ μg protein) was observed (Fig.6).

DISCUSSION

The toxicity of these bloom forming cyanobacteria has been confirmed by biochemical and molecular probes (Pandey, 2007). Our experimental analysis depicted that cyanobacterial strains vary in their growth parameters. The amount of pigments present in these cyanobacterial strains are sufficient for their utilization as therapeutic agents. Phycobilosomes consists upto 60 % of the total soluble protein in cyanobacteria (Bennett and Bogorad 1973). The metabolism of phycobilosomes thus, has significant impact on the physiology of cyanobacteria. The antioxidant system is an important and necessary part of an organism's response to a changing environment. Our results showed the presence of antioxidative enzymes catalase and APX. Most environmental stresses appear to have an oxidative component. In the case of

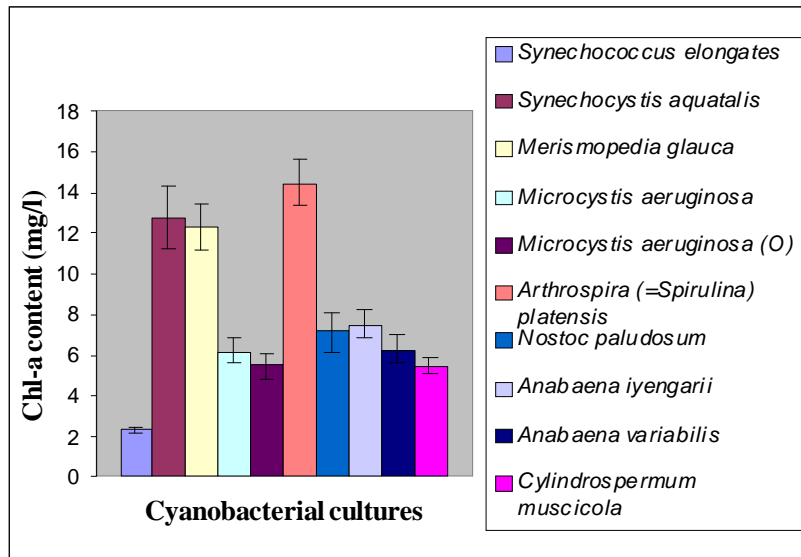


Fig. 3. Chl-a concentration in cyanobacterial cultures.

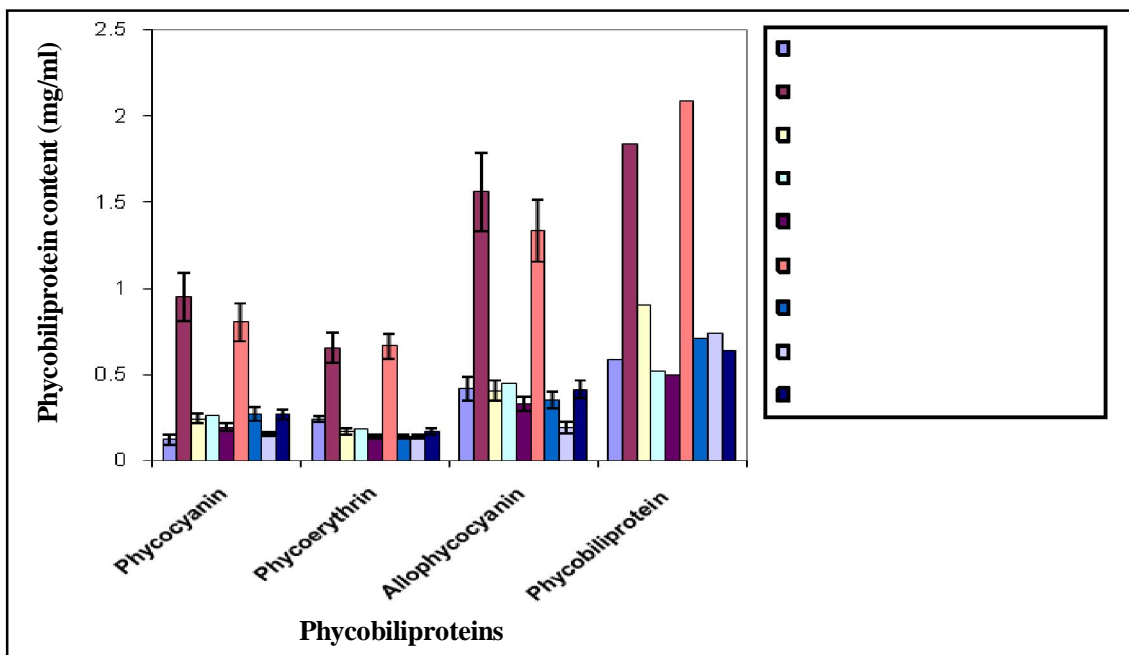


Fig.4. Phycobiliprotein concentration in cyanobacterial cultures.

photosynthetic organisms, antioxidants are needed to adapt to changes in light, temperature and water potential as well as the anthropogenic stresses imposed by herbicides and pollutants. In Cyanobacteria, reports suggested indication of SOD, catalase or some peroxidases under conditions of oxidative stresses based on activity measurements (Mittler and Tel-Or, 1991; Abeliovich *et al.*, 1974). The activity of antioxidative enzymes- Superoxide dismutase, Peroxidase catalase, and Ascorbate Peroxidase has also been reported from marine cyanobacterium *O. willei* BDU 130511 (Saha *et al.*, 2010). The enzymes involved in antioxidative activity and the cellular content of the antioxidants glutathione and ascorbate in the cyanobacteria *N. muscorum* 7119 and *Synechococcus* 6311 has been examined for their roles in

hydroperoxide removal. High activities of ascorbate peroxidase and catalase were found in vegetative cells of both species and in the heterocysts of *N. muscorum* (Tel-Or *et al.*, 1986). Ascorbate peroxidase appears to be a key enzyme in a cascade of reactions regenerating antioxidants. The direct mechanisms to recognize ROS or oxidative damages in cyanobacteria needs to be explored and the mechanisms behind the stability of these enzymes in various systems requires much elucidation.

Conclusion

Cyanobacteria possessed antioxidative enzymes to protect from reactive oxygen species. It was concluded that the antioxidative enzymes- catalase and Ascorbate Peroxidase played a vital role in toxic bloom forming

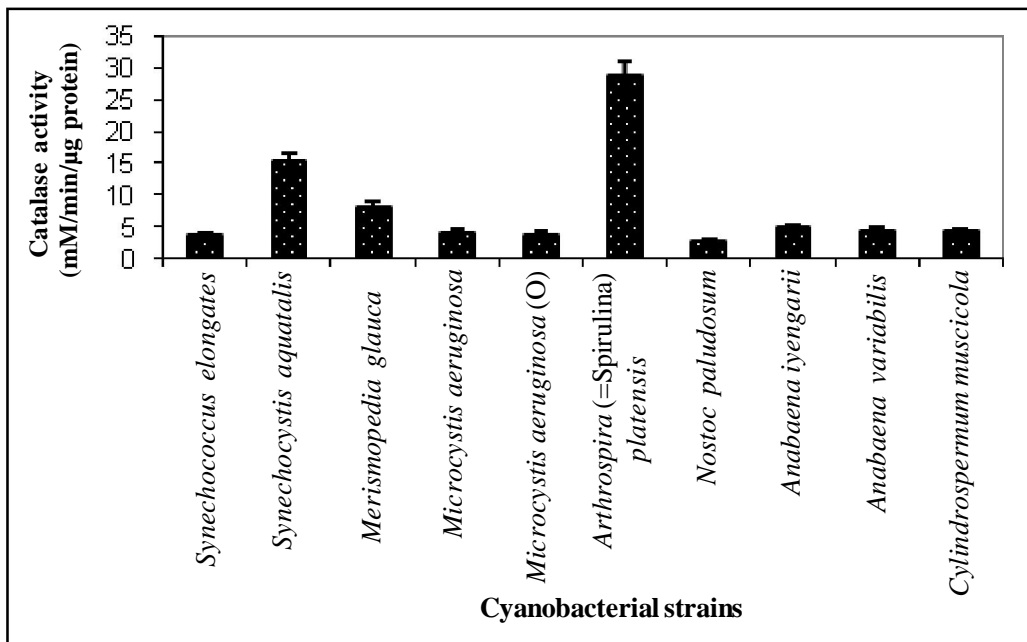


Fig.5. Activity of Catalase in cyanobacterial cultures.

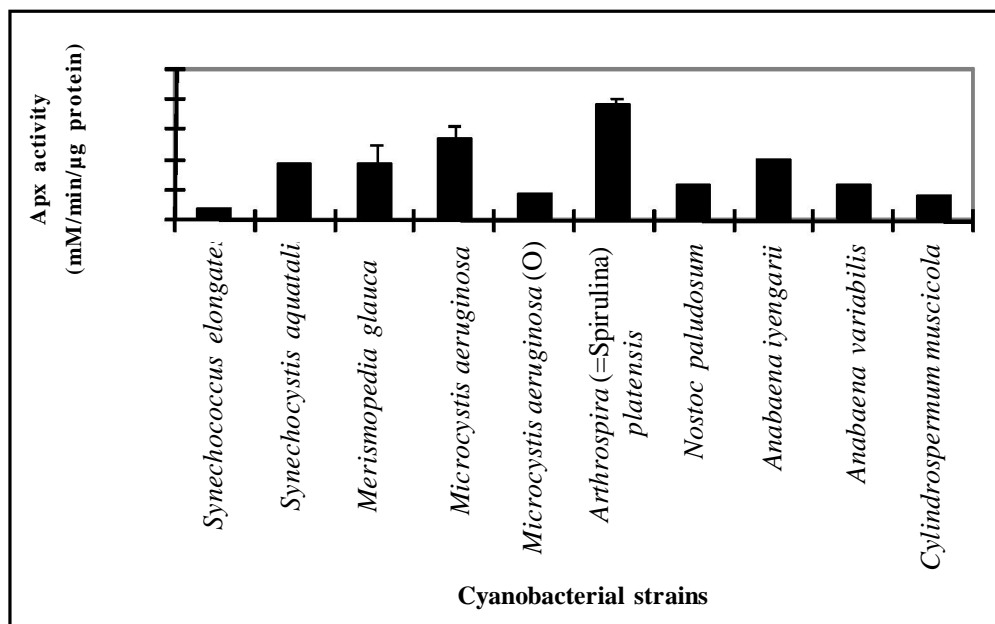


Fig.6. Activity of APX in cyanobacterial cultures.

cyanobacteria. Cyanobacteria are easy to grow; pigments and enzymes can be extracted and employed for various purposes. The antioxidative enzymes of cyanobacteria can be utilized in antioxidative therapy, processing and storage of food and other medicinally important preparations.

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