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Biological Control of Cyanobacteria Using Polyphosphate Accumulating Bacteria Isolated from Activated Sludge

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Increased input of nutrients into water body leads to massive proliferation of cyanobacteria and other algae. This ultimately results in the eutrophication of the water body. The cyanobacterial blooms exert negative environmental impacts such as extensive growth of alga, bad odor, decline in dissolved oxygen, production of cyanotoxin, decreased growth of submerged aquatic organisms and change in taste of water. This results into reduction in qualitative and quantitative properties of water. Various chemical based methods of eutrophication control are suggested by researchers but those endanger the aquatic ecosystem. Considering hazardous effect of chemical agents, biological agents may be used more safely. Here we have shown that polyphosphate accumulating bacteria (PABs) isolated from activated sludge are efficient in reducing cyanobacterial blooms up to 95.56% by trapping phosphorus in metapolyphosphate form. This is the first study that provides direct evidence that use of these microorganisms gives promising result in controlling cyanobacterial blooms and consequently eutrophication process without affecting the ecosystem of water body.

Key words: Eutrophication, Cyanobacterial blooms, Metapolyphosphate accumulating bacteria.

Eutrophication is the process in which clean, clear water resources like lake, pond and even river turns into resources with extensive growth of alga imparting bad odor, decline in dissolved oxygen etc.[1]. It is a growing global problem in large number of inland waters. Survey of the State of the World's Lake showed that 54% lakes in Asia, 53% in Europe, 48% in North America, 41% in South America and 28% in Africa are eutrophic[2].

Although eutrophication is the natural process in aging of lake, human activities can greatly accelerate eutrophication by increasing rate of nutrients and organic substances entering in aqueous system. Agricultural runoff, urban runoff, leaking septic system, water discharges, rain fall, sewage, industrial wastes and similar sources can increase flow of nutrients in aquatic systems. The increased input of nutrients in water body in the form of carbon, nitrogen and phosphorous are considered to be main factors responsible for eutrophication.

Phosphorous is the most important and critical nutrient element responsible for eutrophication than carbon and nitrogen[3]. The eutrophic lake contains 500-1500 mg/m^3 inorganic nitrogen whereas 30-100 mg/m^3 total

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phosphorous[4]. Excess phosphorous causes massive proliferation of cyanobacteria in the water body. Species of *Euglena*, *Anabena*, *Microcystis*, *Oscillatoria*, *Nodularia*, *Nostoc*, *Fragilaria* and *Chlorella* are found to be associated with eutrophic water body[5-7] and are mainly considered as indicators of eutrophication. These cyanobacterial blooms in eutrophic lake exerts negative environmental impacts such as increase in pH, decrease in oxygen content, production of cyanotoxin, decreased growth of submerged aquatic organisms, change in taste and odor of water, thereby making the water body useless for most of the human purposes[8-10]. PABs are found to be efficient in reducing cyanobacterial blooms by trapping phosphorus in metapolyphosphate form. This results into reduction in the eutrophication process without affecting the ecosystem of water body. The overall objective of this research was to control cyanobacterial blooms by PABs isolated from activated sludge.

MATERIAL AND METHODS

Pond selection, determination of its eutrophic status and identification of cyanobacterial flora

The pond having green appearance and dirty smell was selected and studied for types of algae. Number of algal cells per litre was counted microscopically for determination of its eutrophic status[4]. Cyanobacterial scum on water surface and scrapings from floating material were collected in sterile transparent falcon tubes. Identification was carried out microscopically.

Isolation and identification of PABs

PABs were isolated from activated sludge and identified based on biochemical characteristics. Albert's staining method was used to observe polyphosphate granules.

Media optimization

Seven different media namely Gleorge modified medium, Bristons modified medium, Pringsheims medium, Allan and Arnons medium, BG 11 medium, Chues medium and Medium A (sterile water from the studied eutrophic pond + 2% sterile phosphate solution) were tried for media optimization studies.

Effect of PABs on growth of mixed culture of cyanobacteria

Sterile medium 'A' inoculated with mixed cyanobacterial culture (10% v/v) was incubated with isolated PABs (1% v/v, Absorbance of culture suspensions at 480 nm= 0.1). The control was uninoculated with PABs. The incubation was carried out for 15 days at a distance of 15 cm away from the light source (100 W bulb). The growth of cyanobacteria was measured by estimating the chlorophyll content.

Estimation of chlorophyll content for measurement of cyanobacterial growth

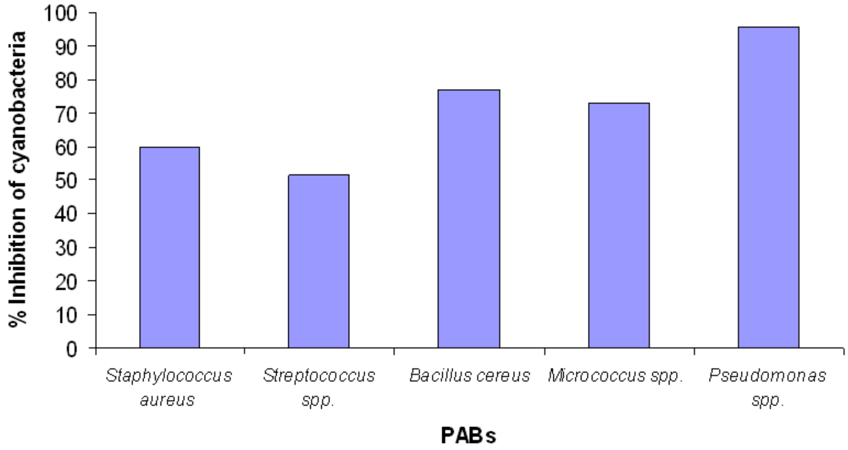
The chlorophyll pigment was extracted by keeping the cyanobacterial growth in 20 ml of acetone for 1 hour. Percent growth and percent reduction of growth was determined by comparing optical density of test with control by spectrophotometric method at 680 nm wavelength.

RESULTS AND DISCUSSION

The selected pond was eutrophic showing more than 20000 cells of cyanobacteria per liter [4]. Most of the strains were pollution tolerant strains from genera *Euglena*, *Chlorella*, *Anabena* and *Microcystis*. Maximum growth of mixed culture of these cyanobacteria was found in Medium A.

Activated sludge samples were investigated for the occurrence of PABs. *Staphylococcus aureus*, *Streptococcus spp.*, *Bacillus cereus*, *Micrococcus spp*. and *Pseudomonas spp.* were found to accumulate phosphate intracellularly. Polyphosphate granules were detected by Albert's staining method and the organisms were identified based on biochemical characteristics. These PABs were found to be efficient in reducing cyanobacterial blooms by trapping phosphorus in metapolyphosphate form, thereby making it unavailable for the growth of cyanobacteria. The reduction in the cyanobacterial blooms was up to 95.56% (Fig 1).

In addition to biological methods other strategies like mechanical removal of blooms, avoiding addition of sources of nutrients to water bodies, pretreatment of nutrient sources before addition into water body and other precautionary strategies should be used for proper control of eutrophication.



Media used: Sterile medium A
Inoculum: Mixed culture of cyanobacteria (10% v/v)
PABs (1% v/v, Absorbance at 480 nm= 0.1)
Incubation Period: 15 days
Light source: 15 cm away from 100 W bulb

Fig. 1. Inhibitory effect of polyphosphate accumulating bacteria on growth of cyanobacteria

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