Ecological Engineering 42 (2012) 107–111

Contents lists available at SciVerse ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Short communication

The importance of bacteria in promoting algal growth in eutrophic lakes with limited available phosphorus

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ARTICLE INFO

Article history:
Received 14 October 2011
Received in revised form 17 January 2012
Accepted 1 February 2012
Available online 4 March 2012

Keywords:
Microcystis aeruginosa
Dissolved organic phosphorus
Dissolved inorganic phosphorus
Growth-promoting effects
Alkaline phosphatase activity

ABSTRACT

Surface waters in Lake Taihu are increasingly threatened by phosphorus (P)-related eutrophication, with a considerable proportion of organic P. A simple experimental model was proposed to characterize "bacterial services" to cyanobacteria. We used the digested solutions of three dissolved organic phosphorus (DOP) compounds (i.e., glucose-6-phosphate, adenosine triphosphate and lecithin) to test the stimulatory effects of nutrient regeneration by bacteria. The growth-promoting effects of six digested solutions on the microalga *Microcystis aeruginosa* (Ma) were studied. Of these six digested solutions, four had significantly growth-promoting effects on Ma, including the digested solutions of the three DOP compounds by bacteria *Gordonia* sp. txj1302RI and glucose-6-phosphate by bacteria *Burkholderia* sp. txj1302Y4. These results demonstrated that bacteria played important roles in decomposing the unavailable organic P into DIP, suggesting that natural bacteria contribute to algal blooms in Lake Taihu.

1. Introduction

Eutrophication is one of the most severe environmental problems, which is caused by the excess phosphorus (P), which serves as a key element limiting primary productivity in aquatic ecosystems (Anderson et al., 2002; Heisler et al., 2008). Surface waters in Lake Taihu are increasingly threatened by P-related eutrophication (Bai et al., 2009; Zhou et al., 2011). In the water of Lake Taihu, the fraction of dissolved reactive P is less than 7% and half of the total phosphorus can be hydrolyzed as inorganic phosphate to compensate for phosphorus deficiency during algae growth (Gao et al., 2006).

For the most part, organically bound P is not directly available to living organisms. To be taken up, organic P should first be converted to orthophosphate (Huang et al., 2005; Song et al., 2009). However, some types of organic P cannot be utilized by alga (Huang et al., 2005; Wang et al., 2011). Moreover, the interaction between bacteria and phytoplankton has been recognized as an important factor in the physiology and dynamics of harmful algal blooms (Gonzalez-Bashan et al., 2000; Liu et al., 2008).

Many studies have focused on the effects of either dissolved organic phosphorus (DOP) or bacteria on cyanobacteria, but there are relatively few studies on the simultaneous effect of both factors. In the present paper, three DOP compounds and two bacterial strains isolated from the Lake Taihu bloom waters were used to study the mechanism of the growth-promoting effects of bacteria on blooming algae in Lake Taihu.

2. Materials and methods

2.1. Algal cultures

The algal strains of *Microcystis aeruginosa* FACHB-912 (Ma) used in this study were isolated from Lake Taihu, China and kindly provided by the Freshwater Algae Culture Collection of the Chinese Academy of Sciences, Wuhan, China.

The growth medium for the algal cultures was BG-11 (Rippka et al., 1979). All stock and experimental cultures were conducted at 25 °C under a 12:12 h (L:D) cycle at approximately 90 μmol photons m^{-2} s^{-1} (Dominguez-Bocanegra et al., 2004).

2.2. Bacterial isolation, maintenance and effects on algal growth

Two bacterial strains, *Gordonia* sp. txj1302RI (RI) and *Burkholderia* sp. txj1302Y4 (Y4), were isolated from the natural freshwater of Lake Taihu. These were incubated at 28 °C for 2 days at 200 rpm and then harvested by centrifugation (3500 rpm, 15 min, 25 °C). The pellets were rinsed three times, suspended in P-free BG11 medium (5 mL) and centrifuged (3500 rpm, 15 min, 25 °C). The

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doi:10.1016/j.ecoleng.2012.02.007

washed pellets were re-suspended in P-free BG11 medium (10 mL). 5 mL sample of each bacterial suspension was added to separate 45 mL P-free BG-11 Ma culture. All flasks were incubated for 25 days under the conditions previously described and monitored every 5 days.

2.3. Preparation of digested solution

2.3.1. Digested solution of DOP

The average total P concentration of Lake Taihu is 0.12 mg L^{-1} but varies significantly from 0.05 mg L^{-1} in Xukou Bay to 0.24 mg L^{-1} in Meiliang Bay (Bai et al., 2009). Thus, the DOP concentration in the experiment was set to 0.12 mg P/L.

The two bacterial suspensions were obtained following the above procedures. The DOP compounds glucose-6-phosphate (G-6-P), adenosine triphosphate (ATP), and lecithin (LEC) (10 mL, 0.2 mg L^{-1}) were added to separate bacterial suspensions (10 mL) and incubated at 28 °C for 3 days at 200 rpm. The incubations were harvested (5000 rpm, 15 min, 25 °C) and filtered. The supernatants were collected as the digested solutions of DOP [DS(DOP)].

The six DS(DOP) were as follows: P-free BG11 medium with the digested solution of ATP by bacterium RI [DS_{RI}(ATP)], P-free BG11 medium with the digested solution of G-6-P by bacterium RI [DS_{RI}(G-6-P)], P-free BG11 medium with the digested solution of LEC by bacterium RI [DS_{RI}(LEC)], P-free BG11 medium with the digested solution of ATP by bacterium Y4 [DS_{Y4}(ATP)], P-free BG11 medium with the digested solution of G-6-P by bacterium Y4 [DS_{Y4}(G-6-P)], and P-free BG11 medium with the digested solution of LEC by bacterium Y4 [DS_{Y4}(LEC)].

2.3.2. The digested solution of P-free medium

Ten milliliters of P-free BG11 medium was added into each of the two bacterial suspensions (10 mL) and incubated at 28 °C for 3 days at 200 rpm. The incubations were harvested (5000 rpm, 15 min, 25 °C) and filtered. The supernatants were collected as the digested solutions of P-free medium [DS(-P)]. The two kinds of DS(-P) were as follows: P-free BG11 medium with the digested P-free solution by bacterium RI [DS_{RI}(-P)] and P-free BG11 medium with the digested P-free solution by bacterium Y4 [DS_{Y4}(-P)].

2.4. Growth kinetics under P-limited cultures

Algal cells were harvested (5000 rpm, 20 min, 20 °C), and the pellet was rinsed three times, suspended in P-free BG11 medium (200 mL) and finally centrifuged (5000 rpm, 20 min, 20 °C). The collected Ma culture was inoculated semi-continuous in 100 mL flasks containing 50 mL of the P-free BG11 medium. Next, 60 μL samples of each of the six DS(DOP) solutions were separately added to each of the flasks. The control flasks were separately treated with 60 μL P-free BG11 medium (-P), 60 μL DS(-P), 60 μL 0.1 mg L^{-1} undigested ATP (ATP), 60 μL 0.1 mg L^{-1} undigested G-6-P (G-6-P), and 60 μL 0.1 mg L^{-1} digested LEC (LEC).

The algal cell densities were determined on days 0, 5, 10, 15, 20, and 25. Total chlorophyll-a (Chl a) was extracted with 90% acetone and determined according to the national standard method (Standard Methods for the Examination of Water and Wastewater, 1998).

2.5. Determination of dissolved inorganic phosphorus and alkaline phosphatase activity

Dissolved inorganic phosphorus (DIP) was determined in triplicate using a SEAL AQ2 discrete analyzer according to the national standard method (Standard Methods for the Examination of Water and Wastewater, 1998; Methods for Chemical Analysis of Water and Wastes, 1983).

Alkaline phosphatase was measured spectrophotometrically as described by Berman (1970). A unit of enzymatic activity was defined as that which gave rise to 1 μg phosphate mL^{-1} h^{-1}.

2.6. Identification of bacteria

The actively growing liquid culture of each isolate was used directly for the PCR reaction. The full-length 16 S rDNA was amplified using the primer pair 8F/1492r (Vickerman et al., 2007). The amplification products were purified, linked and sequenced according to Han et al. (2011). The nucleotide sequences of the isolates were analyzed with the BlastN search program from the National Center for Biotechnology Information website.

2.7. Data analysis

Data were checked for deviations from normality and homogeneity of variance before analysis. An analysis of variance was applied to assess significantly differences between the various treatments using DPS (version 7.05). Pearson correlation coefficients were calculated between all variables measured during incubation. Linear regression was carried out to analyze the relationship among variables.

3. Results

3.1. Effects of the digested solutions on algal growth

The Chl a contents of Ma co-cultured with bacteria RI or Y4 were similar to Ma cultured with P-free medium (-P) (data not shown here). Four of the six digested solutions, specifically, treatments with solutions of three DOP digested by bacterium RI [DS_{RI}(DOP)] and DS_{Y4}(G-6-P) had significantly growth-promoting effects on Ma (Fig. 1A, C–E). Chl a contents in the treatments with DS_{RI} (ATP) were significantly higher than those with DS_{Y4}(ATP) (Fig. 1A and B). The same phenomenon was found in the digested solutions of LEC (Fig. 1E and F). However, the Chl a contents in treatments with DS_{Y4}(G-6-P) were significantly higher than those with DS_{RI}(G-6-P), despite the fact that these two treatments both showed promoting effects on Ma growth (Fig. 1C and D).

All three DS_{RI}(DOP) had similar growth-promoting effects on Ma. Chl a contents increased two to four times compared with the initial values by the fifth day and declined thereafter (Fig. 1A, C and E). Among the three treatments with the DOP compounds digested by bacterium Y4, only one treatment with DS_{Y4}(G-6-P) showed significantly growth-promoting effects on Ma (Fig. 1 B, D and F). During the 25-day incubation period, the Chl a contents in the treatment with DS_{Y4}(G-6-P) were significantly higher than those in the three controls and reached the highest on the 15th day (Fig. 1D, $P < 0.05$).

3.2. Changes in dissolved inorganic phosphorus and alkaline phosphatase activity

Due to the digestion by bacteria, the concentrations of DIP in DS_{RI}(DOP) and the DS_{Y4}(G-6-P) were significantly higher than that in -P during the first 5 days (Table 1, $P < 0.05$). The DIP then declined for the next 10 days. The concentration of DIP of DS_{RI}(LEC) was the highest among the four treatments during the first 20-day incubation period (Table 1).

The initial alkaline phosphatase activity (APA) in the Ma cultures with DOP was one to three times higher than those with