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EUTROPHICATION IN LAKES

Quantitative studies on phosphorus transference occuring between *Microcystis aeruginosa* and its attached bacterium (*Pseudomonas* sp.)

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Abstract Phosphorus release from *Microcystis aeruginosa* and attached bacterium (*Pseudomonas* sp.) isolated from Lake Taihu was examined using a phosphorus isotope tracer in order to investigate the phosphorus transference between the two species. Our results reveal that the amount of phosphorus released form ^{32}P-saturated *M. aeruginosa* is determined by its growth phase and most of phosphorus is assimilated by *Pseudomonas* finally while the amount of phosphorus released from ^{32}P-saturated *Pseudomonas* is also determined by the growth phase of *M. aeruginosa* and most of them are assimilated by *M. aeruginosa*. The results suggest that phosphorus transference occurs between *M. aeruginosa* and its attached *Pseudomonas* . This process makes microenvironment of mucilage of *M. aeruginosa* attached bacteria maintain relative high amounts of phosphorus. Attached bacteria may be a temporary phosphorus bank to the growth of *M. aeruginosa*, and assimilation of phosphorus by *M. aeruginosa* becomes easy when *M. aeruginosa* is in lag growth phase. Thus, the phosphorus exchange between *M. aeruginosa* and attached *Pseudomonas* in microenvironment may be important to microfood web and cyanobacteria bloom.

Keywords *Microcystis aeruginosa* · Attached bacterium · *Pseudomonas* sp. · Phosphorus · Lake Taihu · ^{32}P release

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Eutrophication of shallow lakes with special reference to Lake Taihu, China

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Introduction

Microcystis aeruginosa is the dominant cyanobacterium in Lake Taihu, a shallow hypereutrophic lake in eastern China with extensive cyanobacterial algal blooms in summer and autumn (Dokulil et al., 2000). *M. aeruginosa* forms large mucilaginous colonies, usually colonized by the great deal of bacteria. There is a mutualistic relationship between *M. aeruginosa* and attached bacteria (Whitton, 1973). This symbiotic relation may be favorable to *Microcystis* cells (Steppe et al., 1996). *M. aeruginosa* constitute a microhabitat where attached bacteria are sheltered from grazing by embedding in the mucilage. The microenvironment allows attached bacteria to associate with

the primary producer of organic carbon for its growth. In this microenvironment, heterotrophic bacteria are important: (1) as contributors of CO_2 and possibly sources of nitrogen, phosphorus and trace elements to *M. aeruginosa*; (2) as primary consumers of algal excretions; and (3) as decomposers. Some bacteria may promote blooms while others have algicidal and lysis effects and are involved in termination and decomposition of blooms (Caiola, 1991; Pellegrini et al., 1997; Lovejoy et al., 1998; Van Hannen et al., 1999; Manage et al., 2000; Manage et al., 2001). Thus, the metabolic relationship of *Microcystis* with attached bacteria is important for understanding bloom dynamics.

Phosphorus is the major limiting nutrient for primary producer, including *M. aeruginosa* in Lake Taihu (Dokulil et al., 2000). Pilot studies were conducted on the phosphorus metabolism of *M. aeruginosa* and its attached bacteria, respectively (Shi et al., 2003; Shi et al., 2004; Zou et al., 2005). The information of interaction of cyanobacterium with its attached bacterium for phosphorus transference was little. In this paper, a phosphorus isotope tracer was used to study phosphorus uptake and release from *M. aeruginosa* and its attached bacterium, *Pseudomonas*, to probe their interactions and phosphorus transformation.

Materials and methods

Organism and cultivation

Microcystis aeruginosa was obtained from Institute of Hydrobiology, Chinese Academy of Sciences. It was grown in the modified MA medium (pH = 8.6) in which inorganic phosphorus was replaced by β-Na_2-glycerophosphate (Oh & Lee, 2000). Cultures were incubated at 25°C under illumination intensity of 2200 lx with a 14:10 h of light:dark cycle.

Pseudomonas sp. was isolated from the mucilage of *Microcystis* in Lake Taihu, China and grown in complex medium: glucose 6.0; NH_4Cl 1.0; NaCl 2.0; $MgSO_4{\cdot}7H_2O$ 0.2; K_2HPO_4 1.0; KH_2PO_4 1.0; yeast extract 0.5 (all in grams per liter of deionized water). pH was adjusted to 7.2–7.5 before the complex medium was autoclaved.

Precultivation

This study was conducted with phosphorus-starved *M. aeruginosa*, ^{32}P-saturated *M. aeruginosa*, phosphorus-starved *Pseudomonas* and ^{32}P-saturated *Pseudomonas. M. aeruginosa* and *Pseudomonas* in exponential growth phase were harvested by centrifugation at 10,000*g* for 15 min respectively, washed with sterile deionized water and resuspended in MA medium without phosphorus. Phosphorus-starved cells were directly obtained after incubation for 3 days. Phosphorus-starved cells were collected by centrifugation and transferred to MA medium in which β-Na_2-glycerophosphate was replaced with 12 mg l^{-1} $Na_2H^{32}PO_4$, then were incubated for 3 days to obtain ^{32}P-saturated cells of *M. aeruginosa* and *Pseudomonas* respectively.

Transference of phosphorus from ^{32}P-saturated *M. aeruginosa* to *Pseudomonas*

Attachment of bacteria to *M. aeruginosa* mucilage made it difficult to measure ^{32}P content in bacteria cells or in cyanobacteria cells. Considering this difficulty, a dialysis membrane bag was used to separate *Pseudomonas* from *M. aeruginosa.* However, low molecular weight substances, such as phosphate and small organic compounds, could move across the dialysis membrane.

Phosphorus-starved *Pseudomonas* cells were harvested by centrifugation at 10,000*g* for 15 min, and washed with sterile deionized water and resuspended in a 500 ml beaker containing 200 ml MA medium without phosphorus to 1.0×10^7 cell ml^{-1}. ^{32}P-saturated *Microcystis* cells were harvested by the same way except they were resuspended in the dialysis bag to 5.2×10^6 cell ml^{-1}. This dialysis bag was put in the beaker so then *M. aeruginosa* and *Pseudomonas* cells were separated, while phosphate could migrate across the dialysis membrane. They were incubated at 25°C under a 14:10 h of light:dark cycle.

In order to probe the change of intracellular phosphorus, the cells of *M. aeruginosa* or *Pseudomonas* in certain volume were separated

respectively via centrifugation at 10000 *g* for 15 min and rinsed several times with MA medium until rinse solution radioactivity was close to background. These cells were digested with 0.2 ml 60% $HClO_4$ and 0.4 ml 30% H_2O_2 at 80°C for 60 min. Then, the digested liquids and the upper aqueous solution separated by centrifugation were respectively put into scintillation vials containing 5 ml Triton X-100 toluene scintillator to analyze the radioactivity with a Beckman LS9800 liquid scintillation counter. Actual phosphorus concentration was corrected using the radioactive decay rate. All samples had three replicates.

In order to measure the biomass of *M. aeruginosa* and *Pseudomonas*, their optical densities were measured with at 460 nm and 380 nm, respectively. Then, the amounts of ^{32}P in *M. aeruginosa* or *Pseudomonas* were calculated.

Transference of phosphorus from ^{32}P-saturated *Pseudomonas* to *M. aeruginosa*

The experimental procedures were same as described above except that phosphorus-starved *M. aeruginosa* was incubated in a 500 ml beaker, while ^{32}P-saturated *Pseudomonas* was incubated in dialysis bag.

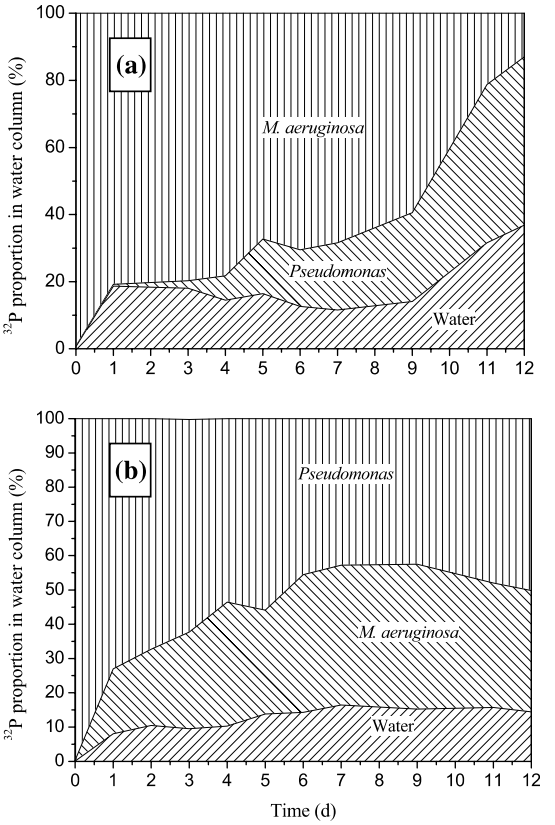

Fig. 1 The transference of ^{32}P released from *M. aeruginosa* (**a**) and from *Pseudomonas* sp. (**b**) among water column.

Results

Transference of phosphorus from ^{32}P-saturated *M. aeruginosa* to *Pseudomonas*

The transference of phosphorus from *M. aeruginosa* to *Pseudomonas* was investigated in a culture system of phosphorus-starved *Pseudomonas* and ^{32}P-saturated *M. aeruginosa* which were separated by a dialysis membrane (Fig. 1a). The growth phases of *M. aeruginosa* comprised four distinct phases of the lag (0–1 day), exponential (1–4 day), stationary (4–6 day) and decline phases (6–12 day) while the growth phases of *Pseudomonas* was in the exponential phase. The total ^{32}P in *Pseudomonas* always increased though the increase was slight for *Pseudomonas* in initial 4-days. Because of the growth of *Pseudomonas* and its incessant assimilation of ^{32}P, the ^{32}P content in *Pseudomonas* increased after *M. aeruginosa* was in the stationary phase. ^{32}P content in *Pseudomonas* increased to 50% of the total ^{32}P on the 12th day when *M. aeruginosa* was in the decline phase.

During the whole experiment period, the intracellular ^{32}P content of *M. aeruginosa* decreased at all times. The ^{32}P concentration in the aqueous solution remained relative steadily from the 1st day to the 9th day, then the sharp increase happened because *M. aeruginosa* in decline growth phase released a large amount of phosphorus to aqueous solution.

Transference of phosphorus from ^{32}P-saturated Pseudomonas to *M. aeruginosa*

Not only phosphorus could move from *M. aeruginosa* to *Pseudomonas*, but also from

Pseudomonas to *M. aeruginosa*. When phosphorus-starved *M. aeruginosa* was present, partitioning of ^{32}P released from ^{32}P-saturated *Pseudomonas* was observed (Fig. 1b). The growth of *M. aeruginosa* exhibited the lag phase (0–2 day), exponential phase (2–9 day) and decline phase (9–12 day) while *Pseudomonas* grew slightly at all times. Most of ^{32}P released from *Pseudomonas* was assimilated by *M. aeruginosa.*

Intracellular ^{32}P content of *M. aeruginosa* increased in the lag and exponential phases, but decreased in decline phase which decrease of ^{32}P content in *M. aeruginosa* was due to the growth of *Pseudomonas* though this decrease was not rapid. ^{32}P concentration in the aqueous solution increased in the first day and then remained relative steadily in the following days.

Discussion

In this experimental system, phosphorus was main factor for the growth of *M. aeruginosa* and *Pseudomonas* and the latter was also controlled by organic carbon. When the intracellular phosphorus content of *M. aeruginosa* was luxury, it grew by utilization of the intracellular phosphorus in its lag and exponential phases (Okada & Sudo, 1982), thus, only 20% of intracellular phosphorus was released form *M. aeruginosa* to aqueous solution and to *Pseudomonas*. However, *M. aeruginosa* released a lot of phosphorus in its stationary and decline phases while it could simultaneously provide *Pseudomonas* with organic carbon and trace elements (Sommaruga & Robarts, 1997; Worm, 1998; Brunberg, 1999). Thus, the growth of *Pseudomonas* was better and the assimilated phosphorus was more when *M. aeruginosa* was in decline phase than that in stationary phase.

After the amount of *M. aeruginosa* was low enough, its second growth became possible. *M. aeruginosa* could use phosphorus in *Pseudomonas* to grow because *Pseudomonas* had a geat deal of phosphorus which was assimilated from overripe *M. aeruginosa* formerly (Fig. 1b). Compared with the phosphorus transference form *M. aeruginosa* to *Pseudomonas*, phosphorus movement form *Pseudomonas* to *M. aeruginosa* was easy and a great deal of ^{32}P was assimilated by *M. aeruginosa* in lag and exponential phases. When *M. aeruginosa* was in the decline phase, its intracellular ^{32}P then turned back to *Pseudomonas* again. This process made soluble phosphorus in aqueous solution stable. Thus, phosphorus released form *M. aeruginosa* could store in attached bacteria and *Pseudomonas* might be a temporary phosphorus bank to *M. aeruginosa* in the microenvironment for its growth.

Conclusion

Phosphorus transference from *Pseudomonas* to *M. aeruginosa* occurs when *M. aeruginosa* is in grow phase while phosphorus transference from *M. aeruginosa* to *Pseudomonas* happens when *M. aeruginosa* is in decline phase. *Pseudomonas* may serve as a temporary phosphorus bank to *M. aeruginosa* in the microenvironment. Thus, phosphorus transference between cyanobacteria and their attached bacteria is decided by their growth phases and phosphorus amount and this exchange could keep an amount of phosphorus in the microenvironment of cyanobacteria and their attached bacteria which is important to microfood web and cyanobacteria bloom.

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