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Monitoring bioavailable phosphorus in lotic systems: A polyphasic approach based on cyanobacteria

M. Ángeles Muñoz-Martín, Aitor Martínez-Rosell, Elvira Perona, Francisca Fernández-Piñas, Pilar Mateo *

Department of Biology, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

HIGHLIGHTS

- Bioanalytical tools are used to analyse actual P bioavailability in running waters.
- Cyanobacterial based P bioreporter and phosphatase activity provide valuable information of the bioavailable P fraction.
- Morphological features may be used to assess P-limitation or P-enrichment in cyanobacteria.
- Bioanalytical data were complementary providing a more complete assessment of nutrient status of fluvial systems.

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ABSTRACT

Conventional assays to measure phosphorus in freshwater systems are sometimes not sufficient to quantify the actual bioavailable P for aquatic biota since some inorganic or organic P species may not be detected by chemical methods, and their bioavailability can be affected by a range of environmental factors. This situation could lead regulatory agencies to be unable to detect imminent ecosystem-degrading phenomena such as cyanobacterial blooms. It could also be an obstacle in studying the ecophysiological requirements of freshwater communities. P bioavailability in five rivers located in central Spain was analysed by a polyphasic approach (combinations of different marker types) based on cyanobacteria. This approach included a parallel study with the use of a self-luminescent P-cyanobacterial bioreporter based on a phosphatase alkaline promoter, determination of *in situ* alkaline phosphatase activities from cyanobacteria found at sampling sites, and the characterisation of cyanobacterial morphological features related to P bioavailability (hairs, polyphosphate granules and calyptras). An inverse relationship was found between values of bioavailable P, measured by the bioreporter and phosphatase activities. Cyanobacteria from sampling sites with low bioavailable P showed high phosphatase activity and *vice versa*, although some differences in values of this activity were observed in different cyanobacteria found at the same place, in relation to different growth strategies. Morphological characteristics associated with P limitation or P enrichment also varied between sampling locations. Cyanobacteria collected from sampling sites with reduced P bioavailability, measured by bioreporter and phosphatase activity, had a lower abundance of polyphosphate granules; those cyanobacteria capable of developing hairs or calyptras showed a greater abundance of these structures. Conversely, polyphosphate granules in cyanobacteria increased as P bioavailability increased as measured by the bioreporter and phosphatase activity. The study shows that the results of genetic, physiological and microscopic analyses based on these methods complement each other, implying that combining their findings would provide a more complete analysis of the nutrient status of running waters.

1. Introduction

Phosphorus is frequently the element that most strongly limits biological productivity in ecosystems. Most organisms take up P from inorganic sources, but organic forms of P often dominate in aquatic ecosystems, which has led to the evolution of complex mechanisms in many organisms that enable them to access P from organic compounds (Turner et al., 2005; Whitton et al., 2005). On the other hand, high P concentrations in waters lead to eutrophication and many associated problems, such as the excessive growth of algae and macrophytes, hypoxia and fish deaths, as well as more frequent cyanobacterial blooms (Dodds, 2006; Hilton et al., 2006). Establishing nutrient criteria has become the goal of national efforts to regulate nutrient content in all water body types in different regions (European Communities, EC, 2000; USEPA, 2000). Since conserving headwater streams and maintaining good water quality are critical for maintaining downstream reservoir water quality, much emphasis has been placed on the quantification

* Corresponding author. Tel.: +34 914978184; fax: +34 914978344.
E-mail address: pilar.mateo@uam.es (P. Mateo).

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of P in running waters. Analysis of P in waters is commonly carried out by colorimetric determination of molybdate-reactive P, which determines the levels of soluble inorganic P and acid-labile organic compounds, so it is sometimes called soluble reactive phosphorus (SRP). Other P fractions, such as dissolved organic phosphorus (DOP), and some non-reactive inorganic polyphosphates are not included in routine analysis (Benítez-Nelson, 2000), leading to a loss of information, which is especially important where aquatic phototrophs are partially or largely dependent on aqueous organic phosphate (McKelvie, 2005; Whitton and Neal, 2011). In addition, the concentration of organic compounds in water can be so low that quantitative analysis is difficult (McKelvie, 2005). Therefore, conventional assays for measuring P in freshwater systems are sometimes not suitable for quantifying the total bioavailable P for aquatic biota.

Bioavailable P, which supports the growth of algae and other organisms, provides the most accurate measure of water quality conditions (Bostrom et al., 1988). However, the actual bioavailability of P under natural conditions varies between freshwaters systems and is affected by a number of environmental factors, such as changes in pH. In addition, the time a certain particle is accessible to algae before sedimentation or washout can affect the actual bioavailability of particulate P (Bostrom et al., 1988). Similarly, organic P can be released from freshwater sediments, whereupon it is hydrolysed within hours to yield a more bioavailable form (McKelvie, 2005).

Cyanobacteria grow abundantly in rivers, and many species are dominant or major functional components in river systems throughout the world, providing the principal energy base for many aquatic food-webs (Scott and Marcarelli, 2012). Due to their ubiquity in aquatic environments and their contribution to total biomass, especially in oligotrophic systems, cyanobacteria can be viewed as a proxy for primary productivity in marine and fresh waters (Bullerjahn et al., 2010). Whereas dissolved inorganic phosphate (DIP) is directly available to cyanobacteria, the degree to which surface-adsorbed P and the complex constituents of the dissolved organic phosphorus (DOP) pool are bioavailable is not accurately known (Dyhrman and Haley, 2006). In cyanobacteria, assays of surface phosphatase activity of field samples and laboratory cultures and correlations between the occurrence of particular taxa and water in which organic phosphate is probably the main form of phosphate, provide considerable evidence of the use of organic phosphates by these organisms (Mateo et al., 2010; Whitton et al., 2005). Alkaline phosphatase is a hydrolase that can remove phosphate groups from many types of ester molecules, such as nucleotides, proteins and alkaloids, and phosphatase activities of cyanobacteria have been proposed as indicators of nutrient status in running waters.

Various morphological characteristics of cyanobacterial cells and filaments are considered excellent environmental indicators, meaning that particular morphological features are associated with P limitation (Berrendero et al., 2008, 2011; Whitton and Mateo, 2012). Long, colourless multicellular hairs, which enhance the surface area for phosphatase activities, are formed by many species, allowing P acquisition from environments where the ambient P concentration is mostly low, but with occasional higher pulses (Whitton et al., 2005; Whitton and Mateo, 2012). Hairs are formed when the average value of cellular P falls to about 1% dry weight, indicating that the organism is P-limited (Whitton and Mateo, 2012).

Most cyanobacteria accumulate polyphosphate (polyP) granules when the cells are P-rich, and these are often large enough to be distinguished easily under the light microscope. Therefore, the relative abundance of polyP granules can also indicate whether an organism is P-limited (Kelly and Whitton, 1998; Whitton and Potts, 2012).

Some Oscillatoriaceae populations (*e.g. Phormidium*) can develop a thickened cap, or an even more elaborate structure, a calyptra, which forms on the outer wall of apical cells (Whitton and Potts, 2012). It has been suggested that the calyptra could be involved in chemotaxis; that such cells form only at one end of the trichome and the terminal regions of these trichomes often flex around suggest that they may play a role in detecting features of their environment such as phosphate or other nutrient gradients, as calyptra development has been found under P-limited conditions (Whitton, 2008; Whitton and Neal, 2011; Whitton and Potts, 2012). An abundance of calyptra might be expected in oligotrophic waters, where P is the limiting nutrient.

Furthermore, bioreporters have been widely acknowledged to represent novel approaches in applied microbiology (Diplock et al., 2010). The development and use of cyanobacterial bioreporters to measure the bioavailability of nutrients may provide valuable information about the fraction of the total P concentration that is actually available to the cyanobacterial cells (Bullerjahn et al., 2010). In a previous study we constructed and characterised several novel cyanobacterial self-luminescent bioreporters of P bioavailability, based on an *Anabaena* sp. PCC 7120, one of which, *A.* AP-L, harbours a fusion of the *phoA*-like promoter to *luxCDABE* operon (Muñoz-Martin et al., 2011). *phoA-like* encodes for an alkaline phosphatase present in many cyanobacteria that is upregulated by P starvation (Gillor et al., 2002; Muñoz-Martin et al., 2011; Ray et al., 1991). Once the *A.* AP-L strain is exposed to P starvation and luminescence induction reaches a maximum, refeeding with increasing P concentrations results in inhibition of luminescence in a concentration–response manner. This may be used to derive a calibration curve with which to quantify the amount of bioavailable P in an environmental sample. It is thus of demonstrable value for monitoring rivers with a wide range of P concentrations (Muñoz-Martin et al., 2011).

We propose in the present paper a polyphasic approach (combinations of marker types) to measure actual P bioavailability to cyanobacteria applied to five Spanish rivers covering a wide range of P concentrations as chemically determined. This approach included a parallel study using the P-cyanobacterial bioreporter based on a phosphatase alkaline promoter, determination of alkaline phosphatase activities and the characterisation of cyanobacterial morphological features related to P bioavailability (*i.e.*, relative abundance of hairs, polyP granules and calyptras). This polyphasic approach illustrates the complementarity of the different methods.

2. Material and methods

2.1. Study area

The study area is located in the central Iberian Peninsula, along the Tajo River basin and its tributaries (Fig. 1A). The Guadarrama and Guadalix Rivers were selected as representative siliceous basin rivers. The Tajo, Gallo and Guadiela Rivers were also studied, since these were examples of calcareous basin rivers. Sampling sites in the Guadarrama and Guadalix Rivers were selected to include locations above and below human settlements and agricultural nuclei. The sampling sites along the Tajo and Gallo Rivers were selected to study the influence of the input of nutrients from the Gallo River into the Tajo River. Finally, the Guadiela River sampling site was selected as an example of a pristine environment. UTM coordinates for sampling sites, ordered upstream–downstream in the Guadarrama and Guadalix River sites, were as follows: Guadarrama 1 (GU1) (30T 408455 4504169); Guadarrama 2 (GU2) (30T 420954 4487373); Guadarrama 3 (GU3) (30T 420817 4474423); Guadalix 1 (GLX1) (30T 436765 4520526); Guadalix 2 (GLX2) (30T 440200 4516000); Guadalix 3 (GLX3) (30T 448370 4504610); Tajo before the Gallo River (TbG), Tajo after the Gallo River (TaG) and the Gallo River (G) (30T 571720 4515053); and Guadiela River (30T 557902 4483479). All sites were sampled in winter 2010 and spring 2011. Water samples were collected in polyethylene bottles and kept cool in darkness. Water aliquots were taken and stored in liquid nitrogen for use in further bioreporter assay experiments.

2.2. Collection and morphological analyses of cyanobacteria

Epilithic cyanobacterial samples were collected at sites where they formed conspicuous mats or colonies (Fig. 1B to I). The

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