Strategies adopted by *Aphanizomenon flos-aquae* in response to phosphorus deficiency and their role on growth

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Abstract

Background: The N₂-fixing cyanobacterium, *Aphanizomenon flos-aquae* is a globally distributed bloom causing species that degrades water quality of freshwater and ocean worldwide. Overcoming phosphorus (P) deficiency is one of the ecological advantages for bloom forming cyanobacteria. It remains unclear to what extent can *A. flos-aquae* alleviate P deficiency by regulating P using strategies.

Results: Based on *in situ* observations of extracellular alkaline phosphatase (APase) in *A. flos-aquae* via enzyme-labeled fluorescence in freshwater bodies in China, Poland and Czechia, we further investigated responses of isolated *A. flos-aquae* to different P supplies (sufficient inorganic P (Pi) as +DIP, α -glycerophosphate and β -glycerophosphate as +DOP _{α} and +DOP _{β} , P-free condition as -P). The significantly negative relationships between percentage of APase producing cells and soluble reactive P concentration in fields and cultures respectively suggested that the excretion of APase was regulated by ambient Pi supply. Suffering from extreme P deficiency in the -P treatments, *A. flos-aquae* showed the highest APase activity reaching a vigorous growth at the early stage, which might also benefit from the formation of polyphosphate body (PPB) and the decrease of cellular P content. In the +DOP treatments, the coordination of dissolved organic P (DOP) mineralization and continuous utilization and formation of PPB might contribute to a maintenance but not reproduction of *A. flos-aquae* when relying on DOP, since the highest cell density reached only 13.38% of that in +DIP treatments while photochemical efficiency was comparable during the whole experiment. Luxury uptake of phosphate as PPB in the +DIP treatments was consistent to the canonical view of polyphosphate as P storage.

Conclusion: *A. flos-aquae* could achieve an instantaneous growth in response to P deficiency with the coordination of P utilization strategies, while only a long-term sustainable growth could be maintained under DOP condition. Persistent and active reproduction could only be achieved in high Pi supply, which implying that an effective consequence can be expected for combating the bloom of *A. flos-aquae* when controlling P supply.

Background

Filamentous cyanobacterial blooms of the diazotrophic genus *Aphanizomenon* occur worldwide and pose deleterious effects on aquatic ecosystem [1, 2, 3, 4]. Theoretically, the nitrogen demand of the developing population of *Aphanizomenon* can be provided by nitrogen fixation in heterocysts [1, 5, 6]. Thus, the phosphorus (P) scarcity can most often lead to its growth limitation. For example, the first appearance of *Aphanizomenon* in Lake Kinneret in August 1994 was apparently elevated by relatively high total dissolved P concentrations [1]. *Aphanizomenon* behaved sensitively when exposed to changes in ambient dissolved inorganic P (Pi) so that it might be adapted to environments with higher P concentrations or repeated interferences of nutrient-enriched water [7]. Orthophosphate in low concentrations led to a strong decrease in the growth of *A. issatschenkoi*, *A. aphanizomenoides* and *A. gracile* [8]. Correspondingly, bloom-forming cyanobacteria developed various strategies to overcome P deficiency. A strategy for cyanobacteria to survive in P deficiency status is that they store P in the form of

polyphosphate bodies (PPB) in cells when P is abundant, and breakdown it upon P stress [9]. Polyphosphate granules observed in cells of *Aphanizomenon* sp. obtained from blooms in Baltic Sea in the summer of 1991 and 1992 suggested that the population was not subjected to P starvation [10]. It also has the ability to decrease cellular P content when P in their environment is scarce [7]. Other studies showed that *Aphanizomenon* could meet its P demand by expressing extracellular alkaline phosphatase (APase), enzymes used to hydrolyze bio-available phosphate from ample dissolved organic P (DOP) molecules [7, 11, 12, 13]. Like the *Aphanizomenon* in Lake Kinneret, whose biomass increased accompanying high enzymatic alkaline phosphatase activity (APA) in phosphate limited summer and autumn [1]. *Aphanizomenon* sp. which is one main bloom-forming filamentous diazotrophic species in Baltic Sea showed level of P deficiency with some percentage of cell-surface-bound phosphatase activity expressed by this population, as indicated by enzyme-labeled fluorescence (ELF) assays [13]. On the contrary, a combination of field and lab-cultures study showed that *A. ovalisporum* excreted APase only under severe P deficiency, it firstly enslaved another phytoplankton other than itself to produce APase [14]. In summary, APase production in the development of *Aphanizomenon* bloom at the single cell level in freshwater is rarely studied. Furthermore, to what extent can *A. flos-aquae* alleviate P deficiency by regulating P using strategies is still unclear.

In this study, single-cell assays of APase were conducted at species level using the ELF technique in 2 Chinese lakes, 1 Czechia reservoir and 1 Polish reservoir. Furthermore, laboratory studies were performed with batch cultures, and three strains of *Aphanizomenon flos-aquae* (*A. flos-aquae* FACHB 1249, *A. flos-aquae* FACHB 1171 and *A. flos-aquae* FACHB 1260) isolated from three Chinese shallow lakes such as Lake Chaohu, Lake Taihu and Lake Dianchi were inoculated in four P status with additions of Pi and two DOP species respectively, as well as without P addition. Multiple variables related to algal growth such as cell density and photosystem II quantum efficiency (F_v/F_m), P cycling such as bulk and size-fractionation APA, concentrations of soluble reactive P (SRP) and DOP in culture media, as well as the ELF labeling and PPB staining of the strains were determined at different intervals. Objectives of this study were 1) to investigate whether *A. flos-aquae* could employ its own APase in acquiring P from DOP for cell growth and reproduction, and 2) to evaluate to what extent can they abate the P limitation status of *A. flos-aquae* together with various P using strategies.

Materials And Methods

Sample collection and chemical analysis

Field samples of surface water (0–50 cm) from different national water bodies were collected for chemical analysis and determination of the algal species and single-cell-bound APase. The sampling water bodies of different nations are marked in the map (Fig. S1). The characteristics of the reservoirs and lakes are shown in Table. S2. Experiments in other countries were completed locally in the cooperative labs.

Experiments in laboratory

Three strains *A. flos-aquae* FACHB 1249 (*A. flos-aquae* 1249 for short), *A. flos-aquae* FACHB1171 (*A. flos-aquae* 1249 for short) and *A. flos-aquae* FACHB 1171 (*A. flos-aquae* 1260 for short) were attained from Freshwater Algae Culture Collection of Institute of Hydrobiology (Wuhan, China). Prior to experiments, cells were harvested in the logarithmic growth period by centrifugation (3000 rpm, 10 min), rinsed 3 times with P-free BG11 medium [15] and then cultivated in P-free BG11 media for 48 h to consume P stored in cells to equal basis [16]. After that, the culture was inoculated in standard BG11 media with modified P concentrations and the modifying set were as follows:

A treatment with no adding P was defined as the -P treatment, in which K_2HPO_4 was replaced with KCl in BG11 media. Two treatments with replete external DOP as the + DOP $_{\alpha}$ and + DOP $_{\beta}$, in which the K_2HPO_4 were replaced by α -glycerophosphate and β -glycerophosphate, respectively. The P concentrations were equal with that in the standard BG11 media. Single addition of sufficient dissolved Pi using the standard BG11 media as the + DIP treatment (the measured P concentration in terms of SRP concentration in standard BG11 media was around $5000 \mu g L^{-1}$).

Initial cell densities of *A. flos-aquae* were roughly 5×10^8 cells L^{-1} . All cultures were incubated in conditions suitable for cyanobacterial growth: constant white light intensity of $30 \mu mol$ photons $m^{-2} s^{-1}$ irradiance, 12 h of light: 12 h of dark, and 25 °C.

Subsamples were collected at different intervals over 59 days for cell density, chemical, enzymatic and DAPI staining of PPB analysis.

Cell density, specific growth rate and photochemical efficiency of photosystem II

Samples for cell density estimation were preserved with Lugol's solution and counted with an Olympus BX 41 microscope (Olympus Corporation, Japan). The specific growth rate was calculated according to the following equation: $\mu = (\ln Ct_2 - \ln Ct_1) / (t_2 - t_1)$, where Ct_2 and Ct_1 are cell densities at times t_2 and t_1 , respectively.

Fv/Fm is one of the chlorophyll fluorescence parameters which reflects the maximum quantum yield of the photochemistry, its value will decrease when stressed by environmental conditions. This indicator is often used to evaluate whether plant is subjected to conditional inhibition [17]. It was examined by Water PAM2100 (Walz, Germany) and determined according to Ting and Owens [18].

P concentrations and specific total P content in cells

Concentrations of SRP in experiments of field and lab-culture were determined according to Murphy and Riley [19]. The total phosphorus (TP) and total dissolved phosphorus (DTP) concentrations were measured following digestion according to the measurement reported by Beattie et al. [20]. DOP concentration was calculated as the difference between DTP and SRP concentrations.

Aliquots of 5 mL from individual cultures were harvested onto 25 mm GF/F glass fiber filters (Waterman, UK) for the measurement of TP in samples to determine the specific total P content of cells according to Vahtera, et al. [12].

Size-fractionation of APA

APA was quantified by fluorometric assay [21]. It was determined in unfiltered sample (APA_T) and in the filtrates through 3.0 μm and 0.45 μm membrane filters ($APA_{<3.0\mu\text{m}}$ and $APA_{<0.45\mu\text{m}}$, respectively). The contribution of APA to the algal ($APA_{>3.0\mu\text{m}}$) and bacterial ($APA_{0.45-3.0\mu\text{m}}$) fractions were calculated as follows: $APA_{>3.0\mu\text{m}} = APA_T - APA_{<3.0\mu\text{m}}$, $APA_{0.45-3.0\mu\text{m}} = APA_{<3.0\mu\text{m}} - APA_{<0.45\mu\text{m}}$, in which $APA_{<0.45\mu\text{m}}$ represent the dissolved phosphatase activity [22].

Fluorescence measurements and microscopic investigations

APase of single-cell was detected using the ELF method. *A. flos-aquae* obtained from field samples and laboratory cultures were detected using ELF® 97 phosphate (Molecular 146 Probes) on the basis of the protocol described in Strojsova et al. [11].

DAPI (4',6-diamidino-2-phenylindole) is a common nucleic acid stain, it could also bind to polyphosphate, DAPI-stained polyphosphate granules show a yellow fluorescence at 526 nm under UV excitation [23, 24]. Fluorescence staining by DAPI (4',6-diamidino-2-phenylindole) was used to examine *A. flos-aquae* for PPB epifluorescence microscopy (Olympus BX51FL) according to the procedure of Bar-Yosef et al. [14].

Statistical analysis

The Pearson correlation test was performed using the SPSS 18.0 package (SPSS, Chicago, IL). The significance of differences among variables was determined via t-test. Differences were considered significant at $P < 0.05$ or $P < 0.01$.

Results

Differences in growth of *A. flos-aquae* in various P supply

The biomass of *A. flos-aquae* was represented by the cell density. At the beginning, the three *A. flos-aquae* strains incubated in the four treatments gained similar biomass increasing from around 5.0×10^8 cells L^{-1} to 1.5×10^9 cells L^{-1} after 8 days (Fig. 1). The cells in the -P and +DIP treatments were then rapidly growing until the day 20 in terms of cell densities and active values of specific growth rate (Fig. 1 A, D; Fig. 2 A, D). What's more, the average specific growth rates in the -P treatments had the highest values among all the treatments in the first 20 days (Fig. 2 A), after that they grew more slowly reaching the peak at day 31 (with cell densities at 4.6 , 6.6 and 6.5×10^9 cells L^{-1} in *A. flos-aquae* 1249, *A. flos-aquae* 1171 and *A. flos-aquae* 1260, respectively), followed by remarkable drop in biomass (Fig. 1 A) together with negative specific growth rates (Fig. 2 A), whereas the growth didn't cease and were at constant positive growth rate

in the +DIP treatments (Fig. 2 D), which made the biomass amassed more and more to be the highest (1.75 , 2.55 and 2.86×10^{10} cells L^{-1} in *A. flos-aquae* 1249, *A. flos-aquae* 1171 and *A. flos-aquae* 1260, respectively) compared with other treatments (Fig. 1).

The lowest biomass was that in the treatments of +DOP $_{\alpha}$ and +DOP $_{\beta}$ with the maximum value at 2.3×10^9 cells L^{-1} for the three strains. Specifically, the maximum biomass in the +DOP $_{\alpha}$ and +DOP $_{\beta}$ treatments were about 50% for *A. flos-aquae* 1249, 30% for *A. flos-aquae* 1171 and 1260 of that in -P treatments. While compared with +DIP treatments, they were 12.42 % and 13.38 % for *A. flos-aquae* 1249, 7.64 % and 8.04 % for *A. flos-aquae* 1171, and 6.1% and 6.66% for *A. flos-aquae* 1260 of that, respectively. Noticeably, the biomass in the +DOP $_{\alpha}$ and +DOP $_{\beta}$ kept increased before day 11, then the cell densities stabilized at a ranging of 1.5×10^9 cells L^{-1} to 2.0×10^9 cells mL^{-1} (Fig. 1 B, C). Moreover, the specific growth rates were also the lowest before day 20 compared with other two treatments, after which they showed down tendencies from the highest at the beginning towards nearly none, with sometimes positive or opposite and very low absolute values near zero (Fig. 2 B, C). All three strains performed generally consistent in these growth parameters.

Photochemical efficiency of photosystem II in different treatments

The photochemical efficiency of photosystem II (F_v/F_m) values remained relatively consistent at 0.400 to 0.538 during the whole incubation time in the +DIP treatments. While in the -P treatments, it showed descending after day 31 from consistent values with that in the +DIP treatments and dropped strongly to very low values at the end of the incubation time (0.014, 0.077 and 0.037 for *A. flos-aquae* 1249, *A. flos-aquae* 1171 and *A. flos-aquae* 1260, respectively). On the other hand, the F_v/F_m values in the +DOP $_{\alpha}$ and +DOP $_{\beta}$ treatments were about 0.1 less compared with that in the +DIP treatments, they had no significant differences among the whole incubation time despite a little decreased after day 35 (Fig. 3).

SRP concentrations in different treatments

For all selected strains, the -P treatments always showed low SRP concentrations in the media (Fig. 4 A). Thus, growth conditions in the -P treatments regarding P supply didn't markedly change during the experiment. The SRP concentrations in the +DOP $_{\alpha}$ and +DOP $_{\beta}$ treatments appeared rapid increasing to the peak at the value of around $3.5 \text{ mg } L^{-1}$ for the first 15 days with the DOP concentrations decreased to near none, then the SRP concentrations stabilized at around 2.7 to $3.0 \text{ mg } L^{-1}$ (Fig. 4 B, C). In the +DIP treatments, the SRP concentrations declined gradually before day 20 and more rapidly thereafter. Moreover, they were decreased into the levels at the limit of detection in the strains of *A. flos-aquae* 1171 and *A. flos-aquae* 1260 (Fig. 4 D).

Expression of APase and its relationship with ambient P

The ELF signals in cells of *A. flos-aquae* were observed from different subtropical and temperate water bodies in Czechia, Poland and China, as well as in the studied strains (Fig. 5). Noticeably, the ambient

SRP concentration was high at 0.681 mg L⁻¹ in Stefanski Reservoir in Poland (Table. S1). In addition, the SRP concentration was negatively relative with the ELFp of *A. flos-aquae* in Lake Chaohu, similar trend was also observed in the cultures among four treatments in the lab-culture (Fig. 6).

In the laboratory experiment, the ELFp exhibited significantly positive relationship with APA_{>3.0μm} ($r=0.7768$, $P<0.01$) (Fig. S2). In the culture media of the -P treatments, APA_{>3.0μm} accounted for the largest part of the APA_T (67.6-70.5%) (Fig. S2), which together with the percentages of cells with ELF labeling (ELFp) were both significantly higher compared to those in the other treatments ($P<0.01$) (Fig. S3, S4). In addition, though the ELFp in +DOP_α and +DOP_β treatments were strongly lower than that in the -P treatments (10.5 to 15.2 - fold greater in -P treatments versus DOP treatments, $P<0.01$), they showed remarkably higher than that in +DIP treatments (17.8 to 25.6 - fold, $P<0.01$) (Fig. S4).

Cellular P content and PPB containing cells in different treatments

There were great differences in specific cellular TP content among the four P treatments, which showed remarkable lower values in the -P treatments compared to that in the other ones ($P<0.01$) (Fig. 7). The polyphosphate granules in the cells showed the P storage in *A. flos-aquae* (Fig. 8). In addition, the percentages of cells with PPB staining (PPBp) in the -P treatments also exhibited the lowest ($P<0.01$) (Fig. 9). What's more, the initial percentages of cells with PPB staining (PPBp) was around 0.05% in -P treatments, and it could be observed PPB containing cells before day 15 (Fig. 9 A), with an up-ward trend began at day 1 or 2 to the highest at day 5, and then went down to the none within strains *A. flos-aquae* 1171 and 1260, meanwhile, the strain *A. flos-aquae* 1249 showed the same pattern with the other two but harbored lesser PPBp, the peak is invisible in the figure (Fig. 9 A). In the +DIP treatments, PPBp increased from day 1 and reached maximum at day 2 then remained constant (85-93%), less variation was observed in PPBp (Fig. 9 D). Additionally, numbers of polyphosphate granules within one cell were also relatively constant in the +DIP treatments (Fig. 8 E and F). Furthermore, the PPBp lessened in the strain *A. flos-aquae* 1171 and *A. flos-aquae* 1260 at day 46 to 74% and 78%, respectively (Fig. 9 D). In the +DOP_α and +DOP_β treatments, the PPBp exhibited fluctuations. In details, the PPB containing cells achieved to the most with percentages comparable to that in the +DIP treatments, but with time incubated, the percentages decreased gradually and sometimes even to extremely low levels (Fig. 9 B, C). Furthermore, number of granules with one cell also obviously fluctuated in +DOP_α and +DOP_β treatments (Fig. 8 A-D).

Discussion

Whether cyanobacteria alleviated P deficiency by producing APase is rarely studied, despite numerous studies have showed that it did so in phytoplankton in freshwater and ocean by evaluating the APA in situ and cultures [25, 26, 27, 28, 29, 30]. The current study illustrated the P utilization adoption of *A. flos-aquae* in response to various P supply and its role in regulating the growth and reproduction. A vigorous growth at the early stage in the -P treatment might be owing to the cooperation of P utilization strategies in *A. flos-aquae*, e.g. producing APase, increase PPB formation and decrease cellular P content.

Unexpectedly, *A. flos-aquae* in the treatment of DOP gave the maximal cell density reaching only 13.38%

of that in replete Pi control, even though the quantity and quality of APase were observed and the substrate monoester was sufficient. No growth rate could be determined, while the photochemical efficiency was comparable to the treatment of + DIP, indicating that *A. flos-aquae* maintained its metabolic activity but not reproduced when DOP was supplied as a sole source of P.

The ELF signals in cells of *A. flos-aquae* in the field and lab-culture and the inverse relationship between the percentage of active cells (or the APA contributed by algal cells) and the SRP concentration indicated that the APase excretion by *A. flos-aquae* was regulated by the ambient P concentration, which was commonly observed so that high APA was recommended as an indicator of P stress or limitation [26, 31, 32]. Unexpectedly, *A. flos-aquae* produced APase where SRP concentration was pretty high, e.g. Stefanski Reservoir in Poland. A possible explanation for these observations might be that the threshold for APase initiation may be strain-specific so as to response to local environmental conditions. Some phytoplankton species would respond to P deficiency at high concentrations of SRP, like the *Prorocentrum minimum*, which began to express APA at a higher SRP concentration than *Chaetoceros neogracile*, *Chlorella autotrophica*, *Isochrysis* sp. and *Tetraselmis chui*. tested using the ELF method [33]. In addition, a repression of the synthesis of APase was observed over an orthophosphate concentration threshold between 0.4 and 1 μM in *Alexandrium Catenella* in the France lagoon [34]. In time-course experiment, the APA was induced at orthophosphate concentration at 0.43 mM for *Alexandrium tamarense* isolated from Hiroshima Bay [35]. These results indicated that the threshold for producing APase is species or strain specific. On the other hand, scientists also referred to APA's contribution of DOP mineralization to phytoplankton P absorption and growth [29, 36]. It was quantified that up to 89% of the total phytoplankton P uptake was represented by APA in Lake Wivenhoe of Australia [37]. An anomaly thriving growth of *A. flos-aquae* in the -P treatments at the first 31 days might attribute to the highest APA in this treatment. *A. flos-aquae* secreted APase and hydrolyzed the DOP released from the cells into Pi, and then swiftly absorbed it into cells as the form of PPB, even though the SRP concentration in the medium was undetectable. Another P source in the -P treatments might be the internal reserves of P at the beginning of the incubation. It was not exhausted during the 48 h pretreatment. The PPB containing cells showed an up-ward trends to the highest at day 5, and down-trend to none at day 11 (Fig. 9A). This pattern of PPB forming was contradicted to the canonical point of polyphosphate as a luxury P storage molecule. In accordance with our study, polyphosphate accumulation was observed in cells of *A. flos-aquae* obtained from a summer bloom, where only small amounts of phosphate were available in the ambient environment, which could help to compete with other microorganisms for phosphate during bloom [38]. In addition, the phytoplankton in the western North Atlantic was enriched in polyphosphate in the P-depleted subtropical Sargasso Sea compared with nutrient-replete temperate waters, proposing that the polyphosphate cycle might produce a feedback loop, which reduces the P export when it is lacking and makes contribution to bio-available P for primary production [39]. Another explanation for this transitory growth is that *A. flos-aquae* decreased the requirement of cellular P and alleviated P deficiency to some extent. The lowest specific cellular TP content was found in this treatment (Fig. 7). Consistently, the N:P ratios and intracellular P demand in *A. flos-aquae* TR183, *Nodularia spumigena* AV1 and *Trichodesmium erythraeum* IMS101 can be remarkable plastic in response to P deficiency [12, 40]. It was, therefore,

inferred that the *A. flos-aquae* utilized some strategies such as the promotion of excreting APase, elevation of PPB formation and reduction of P demand to gain maximum growth at the circumstance of P deprivation. This hypothesis was verified by the field observation in Lake Chaohu that high percentage of ELF labeling was accompanied by high biomass. Our previous work about another N_2 -fixing genus *Dolichospermum flos-aquae* also suggested that it suffered from severe P limitation at the end of the bloom [41].

Unlike the pattern of PPB forming in the -P treatments, it seemed following the classical view as a luxury P storage molecule in the + DIP treatments. The types of PPB may explain this variation. Polyphosphate can be classified as acid-soluble polyphosphate (ASP) and acid-insoluble polyphosphate (AISP). ASP is considered to be used for metabolism and production of DNA and proteins, it can also act as a function of short-term P pool. In contrast, AISP appears to be a form of P storage that could be utilized by the cell for growth when the P concentration is limiting in external environment [42, 43, 44], which enables cell divisions in the absence of any exogenous phosphate [45, 46]. Additionally, this polyphosphate was only utilized by microalgae when phosphate concentration of the waste-water reached a growth limiting level [47]. The three strains of *A. flos-aquae* in the + DIP treatments remained active growth after the lag phase and gave the highest biomass with the numbers of polyphosphate granules within one cell as well as PPBp in constant levels (Fig. 8E, F; Fig. 9D). *A. flos-aquae* appeared to use the storage P to achieve active growth in relation to the positive growth rate after day 46 when the SRP concentrations were decreased into the levels of that in the -P treatments in the strains *A. flos-aquae* 1171 and *A. flos-aquae* 1260, where the PPBp began to lessen at that time (Fig. 9D). The main component of the PPB in the + DIP treatments might be AISP which was used upon P deficient environment.

It is expected that APase might overcome P deficiency together with amply supply of the substrate. The use of glycerophosphate and other DOP gave quite similar growth rates to that obtained with inorganic ones [48, 49, 50]. In contrast to these results, the lowest biomass in the glycerophosphate added treatments was detected, with their growth even not as good as that in the -P treatments with P-free condition. The APA in + DOP $_{\alpha}$ and + DOP $_{\beta}$ treatments were significantly lower than that in the -P treatment. Obviously, APA was not regulated by substrate DOP, which was also observed in the culture of *Microcystis aeruginosa* [30]. Low activity in the + DOP treatments could be resulted from feedback inhibition of APA by the phosphate produced in the glycerophosphate hydrolysis [50]. For example, low levels of external phosphate were sufficient to induce repression of APA in *Gelidium latifolium* [51]. Despite the repression of APA in + DOP $_{\alpha}$ and + DOP $_{\beta}$ treatments, as well as the significantly lower exhibition of ELFp compared with the values in the -P treatments, however, ELFp were strongly higher than that in + DIP treatments (17.8 to 25.6 - fold), irrespective of the strains studied (Fig. S4). Consistently, the *Coccomyxa* isolated from Lakes Plesne and Hromnice in Czechia increased in cell-specific phosphatase activity measured combining the ELF assay with image analysis at low P concentration and the cultures treated with DOP (β -glycerol phosphate), which generated remarkably higher activity than those supplied with the same Pi concentrations [52]. Therefore, APase in *A. flos-aquae* could perform its function of hydrolyzing organic P, as illustrated by significant and positive relationship between the ELFp

and $\text{APA}_{>3.0\mu\text{m}}$ (Fig. S2). Furthermore, the SRP concentrations in the + DOP_α and + DOP_β treatments appeared increasing before day 15 with the expense of the DOP concentrations (Fig. 4B, C), which was an additional evidence that the DOP had been decomposed by the enzyme. On the other hand, it was noted that three strains of *A. flos-aquae* in + DOP_α and + DOP_β treatments showed fluctuations in both numbers of the polyphosphate granules within one cell (Fig. 8A-D) and PPBp (Fig. 9B, C) along the culture period. The PPB in the + DOP treatments might mainly be the ASP, which was rapidly utilized after approximate three days by microorganisms in waste-water [47]. Noticeably, the photochemical efficiency in terms of Fv/Fm values was comparable during the whole experiment in the treatments of + DIP and + DOP. Shortly, the effective DOP mineralization and continuous processes of production and consumption of PPB in the + DOP treatments supported an active and sustainable growth but not reproduction for *A. flos-aquae* in + DOP treatments. Consistent to our observation, *A. flos-aquae* TR183 isolated from Baltic Sea yielded lower biomass in glycerol phosphate culture when the Pi in the media was not depleted than that in Pi-replete condition [12]. This suggested that *A. flos-aquae* is a species that grows better in high Pi pulse supply. The coordination of DOP mineralization and the formation and utilization of PPB in *A. flos-aquae* might contribute to this maintaining growth, which was in accordance with the statement raised by Strojsova et al. [53] that the ability to decompose organic polymers and particles with extracellular phosphatases was beneficial for longer persistence of given population in a Pi-scarce circumstance.

Conclusion

Our findings provided evidence that P utilization strategies in *A. flos-aquae*, including producing APase, increase PPB formation and decrease cellular P content, played an important role in response to P stress. DOP was an important alternative P source for longer maintenance in filamentous cyanobacteria under Pi deficiency. Persistent and active reproduction could only be achieved in high pulse Pi supply, implying that an effective consequence can be expected for combating the bloom of *A. flos-aquae* when controlling P supply.

Declarations

Availability of data and materials

The data sets supporting the conclusions of this article are included within the article and its Supplementary file.

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Contributions

1. Zhou, C. Song, X. Cao, A. Sevcu and Tomasz Jurczak conceived and initiated the project. X. Cao, I. Dolinova, A. Sevcu, T. Jurczak, P. Frankiewicz, A. Wojtal-Frankiewicz and Q. Deng took field samples and chemical analysis. X. Cao and X. Chen designed the culture experiments. X. Chen and L. Wan performed the experiment and analysis. X. Chen, Y. Zhou, X. Cao, and C. Song analyzed the data and composed the manuscript. All authors analyzed the results and edited the manuscript.

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Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Additional information

Supplementary file. Additional figures and table.

Abbreviations

P: phosphorus

Pi: inorganic P

PPB: polyphosphate body

APase: alkaline phosphatase

DOP: dissolved organic P

APA: alkaline phosphatase activity

ELF: enzyme-labeled fluorescence

Fv/Fm: photosystem II quantum efficiency

A. flos-aquae 1249: *Aphanizomenon flos-aquae* FACHB 1249

A. flos-aquae 1171: *Aphanizomenon flos-aquae* FACHB 1171

A. flos-aquae 1260: *Aphanizomenon flos-aquae* FACHB 1260

ELFp: percentages of cells with ELF labeling

PPBp: percentages of cells with PPB staining

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Figures

Figure 1

Time series of cell density of *A. flos-aquae* 1249, *A. flos-aquae* 1171 and *A. flos-aquae* 1260 in -P treatments (A), +DOP α (B), +DOP β treatments (C) and +DIP treatments (D). Error bars represent standard deviation of triplicate samples. Different scales for vertical axes in A, B/C and D.

Figure 2

The specific growth rate of *A. flos-aquae* 1249, *A. flos-aquae* 1171 and *A. flos-aquae* 1260 in -P treatments (A), +DOP α (B), +DOP β treatments (C) and +DIP treatments (D). Error bars represent standard deviation of triplicate samples. The numbers above day 20 mean the average specific growth rate during 20 days of *A. flos-aquae* 1249, *A. flos-aquae* 1171 and *A. flos-aquae* 1260 in different treatments.

Figure 3

Photochemical efficiency of photosystem II (F_v/F_m) of *A. flos-aquae* 1249 (A), *A. flos-aquae* 1171 (B) and *A. flos-aquae* 1260 (C) in -P treatments, +DOP $_{\alpha}$, +DOP $_{\beta}$ treatments and +DIP treatments. Error bars represent standard deviation of triplicate samples.

Figure 4

The P concentrations of *A. flos-aquae* 1249, *A. flos-aquae* 1171 and *A. flos-aquae* 1260 in -P treatments (A), +DOP α (B), +DOP β treatments (C) and +DIP treatments (D). Error bars represent standard deviation of triplicate samples.

Figure 5

A. flos-aquae treated with ELF reagent for extracellular alkaline phosphatase (APase) detection. Green ELF fluorescence associated with *A. flos-aquae* obtained from Orlík Reservoir in Czechia (A) and Stefanski Reservoir in Poland (B), shallow lakes of Lake Chaohu (C) and Lake Zhiyinhui (D), as well as the culture strains of *A. flos-aquae* 1249 (E), *A. flos-aquae* 1171 (F) and *A. flos-aquae* 1260 (G) were observed. Scale bars indicate 10 μm.

Figure 6

Relationships between the percentages of cells with ELF labeling (ELFp) in *A. flos-aquae* and the SRP concentrations in Lake Chaohu (A) and in lab-culture (B).

Figure 7

Box plots of temporal variations in the specific cellular TP content in *A. flos-aquae* 1249 (A), *A. flos-aquae* 1171 (B) and *A. flos-aquae* 1260 (C) in -P treatments, +DOP $_{\alpha}$, +DOP $_{\beta}$ treatments and +DIP treatments. The × from the two sides of the column are the minimum and maximum of ELFP, the full lines represent 25% percentiles of the variables, median values and 75% percentiles of the variables. The small squares within the column are their averages. Asterisks (*) indicate significant difference ($P < 0.01$) between represented treatments of the columns and the other treatments.

Figure 8

Polyphosphate body (PPB) detection in *A. flos-aquae* in fluorescence microscope (only shown the images of *A. flos-aquae* 1171). Chlorophyll fluorescence is red and green-yellow fluorescence mark DAPI-stained PPB. Number of polyphosphate granules within cell varied in the +DOP α treatment (A, B), and in the +DOP β treatment (C, D), it showed almost constant in the +DIP treatment (E, F). Scale bars indicate 10 μ m.

Figure 9

The percentages of cells with PPB staining (PPBp) in *A. flos-aquae* 1249, *A. flos-aquae* 1171 and *A. flos-aquae* 1260 in -P treatments (A), +DOP α (B), +DOP β treatments (C) and +DIP treatments (D). Error bars represent standard deviation of triplicate samples.