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Original article

Accumulation of intra-cellular polyphosphate in *Chlorella vulgaris* cells is related to indole-3-acetic acid produced by *Azospirillum brasilense*

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This study is dedicated to the memory of the Israeli soil microbiologist Prof. Yigal Henis (1926–2010) of the Faculty of Agriculture, The Hebrew University of Jerusalem in Rehovot, Israel, one of the pioneers of *Azospirillum* research.

Abstract

Accumulation of intra-cellular phosphate, as polyphosphate, was measured when the microalga *Chlorella vulgaris* was immobilized in alginate with either of two wild-type strains of the microalgae growth-promoting bacterium *Azospirillum brasilense* or their corresponding IAA-attenuated mutants. Wild type strains of *A. brasilense* induced higher amounts of intra-cellular phosphate in *Chlorella* than their respective mutants. Calculations comparing intra-cellular phosphate accumulation by culture or net accumulation by the cell and the amount of IAA that was produced by each of these strains revealed that higher IAA was linked to higher accumulations of intra-cellular phosphate. Application of four levels of exogenous IAA reported for *A. brasilense* and their IAA-attenuated mutants to cultures of *C. vulgaris* enhanced accumulation of intra-cellular phosphate; the higher the content of IAA per culture or per single cell, the higher was the amount of accumulated phosphate. When an IAA-attenuated mutant was complemented with exogenous IAA, accumulation of intra-cellular phosphate at the culture level was even higher than phosphate accumulation with the respective wild type strains. When calculating the net accumulation of intra-cellular phosphate in the complementation experiment, net intra-cellular phosphate induced by the IAA-attenuated mutant was completely restored and was similar to the wild strains. We propose that IAA produced by *A. brasilense* is linked to polyphosphate accumulation in *C. vulgaris*.

Keywords: *Azospirillum*; *Chlorella*; Indole-3-acetic acid; Phosphorus uptake; Plant growth-promoting bacteria; Polyphosphate

1. Introduction

Chlorella spp. are common aquatic unicellular, non-motile, green microalga. Inorganic phosphate (as PO_4^{3-}, Pi) is one of the optimal phosphorus sources for *Chlorella* spp. [1,2]. Assimilation of P in plants happens via an initial step of transporting P in the form of orthophosphate across the plasma membrane of cells. This energy-fueled process is mediated by plasma membrane transporters, which specifically co-transport Pi and protons. The protons generated by a plasma membrane proton pump (H^+/ATPase) is the process for inorganic phosphate uptake [3–5]. This H^+-ATPase transport system generates the electrochemical gradient across the membrane which allows H^+/Pi (proton/inorganic phosphate) co-transport to occur via several carrier proteins. There are two types of phosphate transporters. (1) Low-affinity phosphate transporters with continuously expressed proteins. (2) High-affinity transporters with expression that is regulated and induced when the cells are under phosphate-limiting conditions [3,6]. After the initial assimilation of Pi and under high availability

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of Pi, the proton extrusion mechanism of the cell is activated to balance the excess of protons across the membrane [4,7–9].

Azospirillum spp. are highly motile [10,11], rhizosphere competent [12], plant growth-promoting bacteria (PGPB); most strains are known to significantly enhance plant growth, including *Chlorella* spp. [13,14] and none are phytopathogens [15]. Promoting growth of plants with *Azospirillum* spp. involves many simultaneously operating mechanisms, in tandem or cascading, many only on a small scale, a process termed "Multiple Mechanisms Theory" [16]. Two main mechanisms are production of large quantities of the phytohormone indole-3-acetic acid (IAA) [17–19] and enhancement of uptake of minerals, including phosphates [20–23].

Although uptake and assimilation of Pi in plants and microalgae are well documented [2,4], only a small amount of information is available on any metabolic and growth processes induced by auxins in microalgae [13,24–27]. While the relationship of IAA to promoting plant growth was demonstrated for several PGPB, including *Azospirillum* spp. [28–33] and to *Chlorella* spp. [13,19], the relation between the capacity of a PGPB to produce IAA and effects on P metabolism is not.

Immobilization of microalgae in polymers is a common technical strategy in several biotechnological and bioremediation applications [34–36], where the natural alginate polymer is the most common [37]. Immobilizing *Azospirillum brasilense* or other bacteria with *Chlorella* spp. within the same polymer bead restricts the movements of the PGPB and enhances physiological and biochemical interactions between the two microorganisms, thereby producing a synthetic mutualism [13,38–47].

Our hypothesis is that IAA produced by *A. brasilense* is a measurable factor in phosphate uptake and assimilation in *Chlorella vulgaris*. To test this hypothesis, we measured the effect of two IAA-attenuated mutants of *A. brasilense* on intracellular phosphate accumulation in microalgal cells vs. induction of accumulation of intra-cellular phosphate by their intact parental wild-type strains and IAA-attenuated strains complemented with exogenous IAA to reach the same level of IAA as in the wild type parental strains and measuring the effects of the combination (bacteria–exogenous IAA) on phosphate accumulation compared to the parental strains.

2. Materials and methods

2.1. Microorganisms

C. vulgaris Beijerinck (UTEX 2714, University of Texas, Austin, TX) and the wild-type strain *A. brasilense* Sp245 (Empresa Brasiliera de Pesquisa Agropecuária, EMBRAPA, Rio de Janeiro, Brazil) and *A. brasilense* Sp6 [48] and their IAA-attenuated mutants *A. brasilense* FAJ0009 [29] and *A. brasilense* SpM7918 [48] were used in all experiments. *C. vulgaris* was cultured for 6 days in mineral growth media (C30) [49] at 150 rpm in an orbital shaker at 28 ± 1 °C, and 80 μmoles photon m^{-2} s^{-1}. *A. brasilense* strains was cultured for 17 h in nutrient broth (NB; #N7519, Fluka, Sigma–Aldrich, St. Louis, MO) at 35 ± 2 °C and 140 rpm. Cultures of the two mutants were supplemented with 25 μg L^{-1} kanamycin (#60615, Sigma–Aldrich).

2.2. Immobilization of microalgae and bacteria in alginate beads

Microorganisms were immobilized, as described in de-Bashan et al. [50], where 40 mL *C. vulgaris* culture (6.0×10^6 cells mL^{-1}) was mixed with 160 mL of a sterile, 2000 cP 3% alginate solution, and stirred for 15 min. Using an automatic bead maker, this mixture was dropped into a 2% $CaCl_2$ solution under slow stirring [36]. The beads were stabilized for 1 h at 28 ± 1 °C and washed in sterile saline solution. *A. brasilense* (approximately 1.0×10^9 CFU mL^{-1}) was immobilized similarly. Immobilization normally reduces the number of microorganisms in the beads; therefore, a second incubation was necessary (10% NB, 24 h) [51]. To combine both species in the same beads, a similar procedure was performed, using 20 mL of each culture in a mixture (40 mL total). After the second incubation, the beads were rinsed three times in saline solution (0.85% NaCl), placed in 500 mL Erlenmeyer flasks (40 g of beads per flask) containing 200 mL synthetic growth medium (SGM) containing 25 mg L^{-1} NH_4Cl (described in de-Bashan et al. [41]). The flasks were placed on an orbital shaker for 2 d under the same conditions for culturing *Chlorella*.

2.3. Counting microorganisms after treatment

After each experiment (48 h incubation time), beads containing microorganisms were dissolved in 4% sodium bicarbonate solution at room temperature (~28 °C) for ~30 min. *C. vulgaris* was counted under a light microscope with a Neubauer hemocytometer [13] connected to an image analyzer (Image ProPlus 4.5, Media Cybernetics, Silver Spring, MD). *A. brasilense* was counted under a fluorescent microscope, using a fluorescein diacetate stain (#F7378, Sigma–Aldrich) [52].

2.4. Application of exogenous IAA to cultures of C. vulgaris

For each experiment we used freshly prepared IAA (#I2886-25G, Sigma) dissolved in absolute ethanol and maintained in the dark at 4 °C for the duration of the experiment. The concentration of exogenous IAA (in ng μL^{-1}) was 44.53 (*A. brasilense* Sp245), 51.18 (*A. brasilense* Sp6), 6.96 (*A. brasilense* FAJ0009), and 15.53 (*A. brasilense* SpM7918), corresponding to analyses of IAA done in 2008 [19]. Current analyses of the same strains are presented in the Results Section. These concentrations were applied in quantities of <100 μL $culture^{-1}$, depending on the final concentration needed per treatment. The SGM has a strong buffer capacity. Addition of these quantities of IAA did not change the pH of the medium, which remained stable during the experiment.

2.5. Analytical methods

2.5.1. Phosphate determinations

2.5.1.1. In cultures. Phosphate was analyzed calorimetrically, based on the formation of a phospho-molybdate complex by the interaction between ammonium molybdate and ortho-phosphate in the presence of antimony [53] and adapted to microplates [54].

2.5.1.2. Intra-cellular phosphate. Analysis was based on the method described by Lin and Kao [55], with modifications, as follows: After dissolving the beads, the cells were rinsed 10 times (0.85% NaCl, 4500 × *g*) and stored at −80 °C. Frozen samples of cells were macerated with pestle and mortar using acid-washed glass beads. To the macerate, 20 mL of 0.03 mM H_2SO_4 at pH 3.5 were added and then macerated again. The suspensions were centrifuged at 19,500× *g* for 15 min at 4 °C and phosphate was measured in the supernatant, as described above. Since there is no practical way to discern the contribution of each partner in the co-immobilized assemblage, intra-cellular phosphate of co-immobilized microalgae was calculated by subtracting the intracellular phosphate produced by the microalgae or a bacterial strain when cultivated alone from the intracellular phosphate measured in the co-immobilized treatment. Calculation for per cell content was done by dividing these values by the number of developing cells at specific sampling times. In contrast to microelectrode measurements of ion transport in cells [56], this method cannot differentiate between vacuolar and cytoplasmic phosphate. Phosphate measurements reported here is the sum of both. Net intra-cellular phosphate accumulation was calculated by subtracting the intra-cellular phosphate produced by *C. vulgaris* or any of the bacterial strains from the total intra-cellular phosphate produced by co-immobilizing bacteria and microalgae.

2.5.2. Quantification of polyphosphate and DAPI staining

C. vulgaris immobilized alone and *C. vulgaris* immobilized with *A. brasilense* wild-type strain Sp245 or the corresponding IAA-attenuated mutant FAJ0009 were cultured for 48 h, as described above. At 0, 48, and 72 h, samples of beads were dissolved and the resulting cell suspensions were stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; #D9542-5MG, Sigma), following the method of Eixler et al. [57], with small modifications. Cell suspensions were fixed with 2% glutaraldehyde; aliquots of 5 μL were transferred to new glass slides and DAPI (20 μg mL^{-1}) dissolved in distilled water was added. The presence of polyphosphate granules in *C. vulgaris* was demonstrated by epifluorescence microscopy (BX41, Olympus, Tokyo, Japan). DAPI-stained polyphosphate granules have a yellow fluorescence at 526 nm under UV excitation, using the U-MWU2 filter (Olympus). Polyphosphates were quantified by the method of Eixler et al. [58].

2.5.3. IAA determination in cultures of A. brasilense

Measurement of the amount of IAA produced by the pure cultures of the strains of *A. brasilense* was determined as follows: cultures of each strain (25 mL, 10 replicates per strain) were incubated in OAB medium composed of (in g L^{-1}): KOH, 4.8; malic acid, 5; NH_4Cl, 0.5; NaCl, 1.2; $MgSO_4.7H_2O$, 0.25; K_2HPO_4, 0.13; $CaCl_2$, 0.22; K_2SO_4, 0.17; Na_2SO_4, 2.4; $NaHCO_3$, 0.5; Na_2CO_3, 0.09; $Fe_{(III)}$EDTA 0.07; and (in mg L^{-1}): $MnCl_2 \cdot 4H_2O$, 02; H_3BO_3, 0.2; $ZnCl_2$, 0.15; $CuCl_2 \cdot 2H_2O$, 0.2; $NaMoO_4 \cdot 2H_2O$, 2 and supplemented with tryptophan (200 μg mL^{-1}). Antibiotics (listed above) were also added to the media used for the mutant strains. The cultures were incubated for 48 h under the conditions previously described, then centrifuged for 10 min at 1400 × *g*. The supernatant was transferred to clean test tubes and the IAA was measured by an HPLC (Agilent 1100; Agilent Technologies, Santa Clara, CA). Chromatograms were analyzed and recorded with the software HPCHEM integrating software (G2170BA, Agilent). The HPLC system was equipped with a reversed phase column (TSKgel ODS-120A, 5 μm particle size, 150 × 46 mm; Supelco, Bellefonte, PA) and was run isocratic (all analyses used the same ratio of solvents), using the methanol:water:acetic acid ratio (36:64:1 v/v) as the mobile phase. Injection volume was 100 μL and flow rate was 0.5 mL min^{-1}. The wave length for detection was 290 nm. The standard was IAA (I5148, Sigma).

2.6. Experimental design and statistical analysis

Several variations were used, depending on the specific experiment. Each combination is listed in the respective section of the results: (1) *Azospirillum* alone, (2) *Chlorella* alone, (3) co-immobilized *Chlorella* and *Azospirillum*, (4) immobilization of each mutant of *Azospirillum*, and (5) co-immobilization of *C. vulgaris* with each mutant. Control beads without microorganisms were used. Because growth of *C. vulgaris* fluctuated among different repetitions of the same experiment [59], *C. vulgaris* populations were routinely counted in each experiment, and the calculated values were derived from specific experiments, but each population count in each experiment are not shown because they were previously published [41,42], doubling time under the influence of IAA was calculated from the raw data (http://www.doubling-time.com/compute.php, accessed March 9, 2015). Each experiment with *C. vulgaris* was performed as five replicates, where one Erlenmeyer flask served as a replicate. Each experiment was repeated at least three times and the mean data of all experiments were used for statistical analysis by the Kruskal–Wallis non-parametric analysis at $p < 0.05$, using Statistica 8.0 software (StatSoft, Tulsa, OK).

3. Results

3.1. Accumulation of intra-cellular phosphate in C. vulgaris induced by A. brasilense

Comparing accumulation of intra-cellular phosphate induced by each wild-type *A. brasilense* and their IAA-attenuated mutants after incubation for 48 h, both wild-type strains (Sp6 and Sp245) induced more intra-cellular

phosphate than their respective mutants (SpM7918 and FAJ0009). After incubation for 72 h, only strain Sp6 induced more intra-cellular phosphate than its respective mutants SpM7918 (Fig. 1a). When intra-cellular accumulation of phosphate was calculated per cell, both wild types accumulated more phosphate at 48 h and 72 h of incubation. However, the quantity of accumulated phosphate after 72 h was less than at 48 h (Fig. 1b). Comparing accumulation of intra-cellular phosphate and the level of IAA produced by each of these strains showed that when IAA was higher, intra-cellular phosphate was also higher (Fig. 1c). When the net accumulation of phosphate was calculated, the wild-type stains always led to more accumulated phosphate in *Chlorella* than the related IAA-attenuated strains; however, quantities were always lower after 72 h (Fig. 1d).

3.2. Quantification and visual identification of intra-cellular polyphosphate accumulated in C. vulgaris

Accumulation of polyphosphates inside *C. vulgaris* was highest in cells that were co-immobilized with wild-type strain *A. brasilense* Sp245. Intermediate levels occurred in *C. vulgaris* that were co-immobilized with the mutant strain *A. brasilense* FAJ0009 and the lowest quantity of polyphosphate was generated by *C. vulgaris* when immobilized alone. In the latter case, polyphosphate could be detected and quantified only after incubation for 72 h (Fig. 2a). DAPI-staining of immobilized and co-immobilized cultures of *C. vulgaris* with *A. brasilense* (both wild types and their mutants) revealed extensive staining for polyphosphates inside the cells, while cells of *C. vulgaris* cultivated alone showed no visible staining (Fig. 2b–e).

3.3. Effects of different levels of exogenous IAA on phosphate uptake and accumulation of intra-cellular phosphate by C. vulgaris

Quantification of IAA produced by the four *A. brasilense* strains by HPLC revealed very similar quantities, compared with the analysis of IAA from 2008 [19] that served as a guideline for this study. IAA quantities were (in ng μL^{-1}): *A. brasilense* Sp245, 39.061 ± 2.367; *A. brasilense* Sp6,

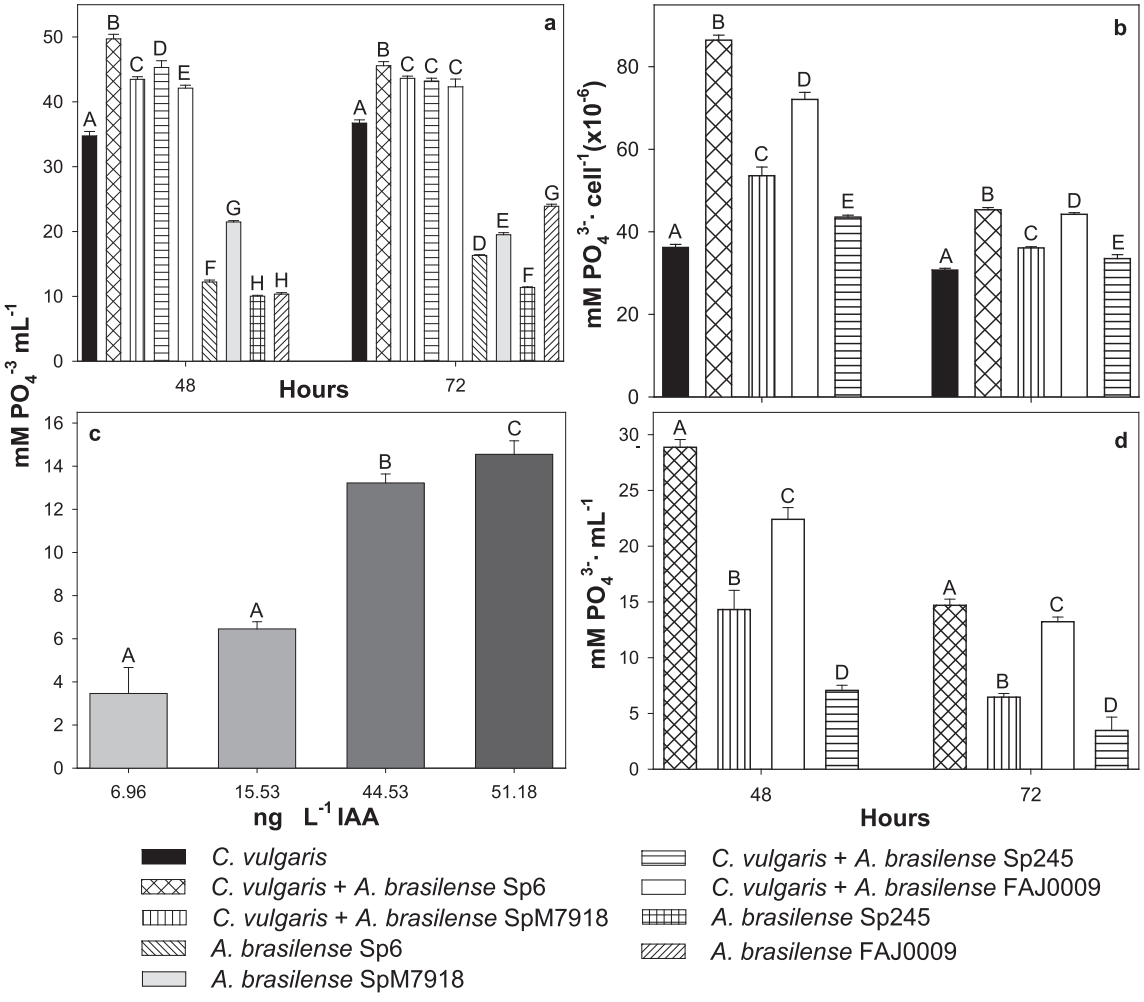

Fig. 1. Accumulation of intracellular phosphate in *Chlorella vulgaris* induced by two wild type strains of *Azospirillum brasilense* and their IAA-attenuated mutants. Accumulation per culture (a) or per cell (b). Calculation comparing between intracellular phosphate accumulation and the level of IAA produced by each of these strains (c). Calculation of net intracellular phosphate accumulation (d). Groups of columns (4 or 9), in each subfigure separately denoted by different letters differ significantly by one-way ANOVA and Tukey's HSD post-hoc analysis at $p < 0.05$.

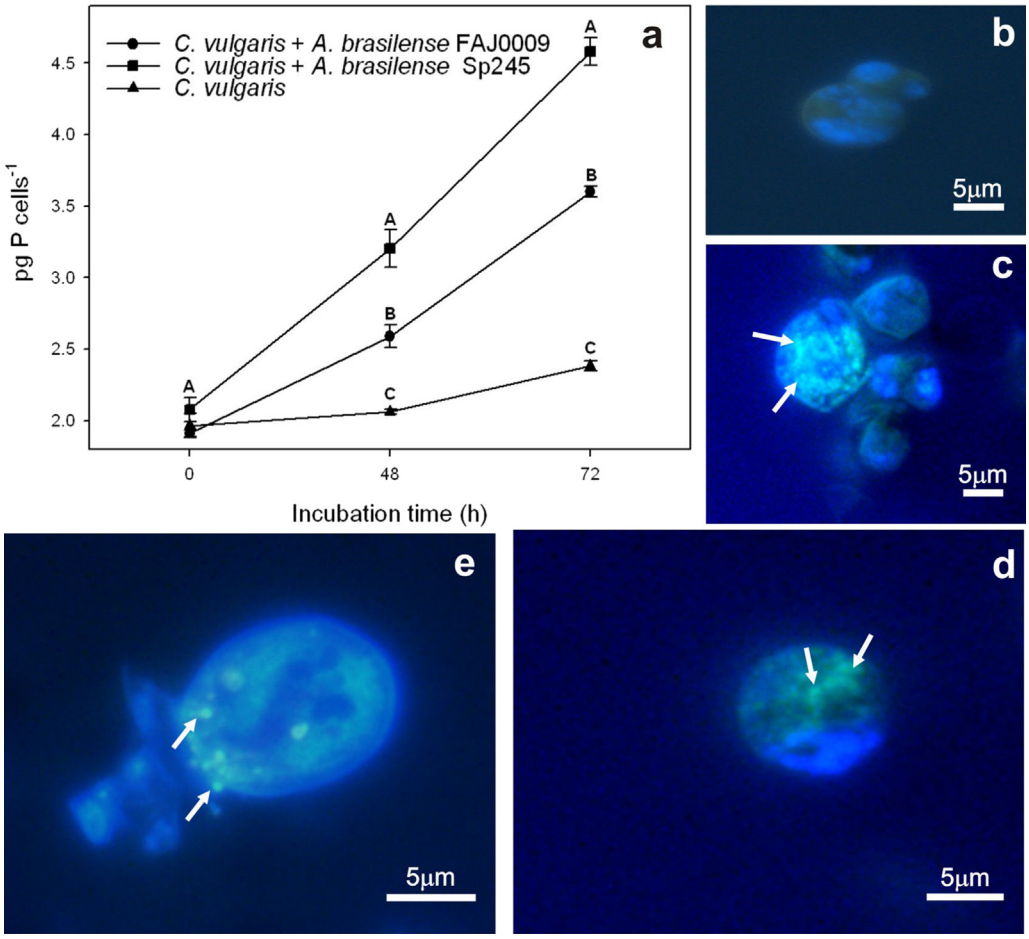

Fig. 2. Quantification of polyphosphates (a) and DAPI staining of polyphosphates under fluorescent microscope in *Chlorella vulgaris* immobilized alone (b) and co-immobilized with *Azospirillum brasilense* Sp245 (c, d) and its mutant *A. brasilense* FAJ0009 (e) in alginate beads.

56.779 ± 2.032; *A. brasilense* FAJ0009, 4.122 ± 0.037; *A. brasilense* SpM7918, 13.753 ± 0.65.

When four different concentrations of exogenous IAA in *A. brasilense* and its IAA-attenuated mutants were tested with *C. vulgaris*, accumulation of intra-cellular phosphate was greater when the quantity of IAA in the trial was greater. This effect was stronger when incubated for 48 h than for 72 h (Fig. 3a). Growth of *C. vulgaris* corresponded to the level of IAA that was applied, with lower enhanced growth induced by the lower levels of IAA in the two mutants than by wild types (data not shown). A similar pattern of intra-cellular phosphate occurred when accumulation was calculated on a per cell basis (Fig. 3b). Doubling time (in h) of *C. vulgaris* was: *C. vulgaris* alone, 57.83; *C. vulgaris* + 6.96 ng L^{-1} IAA, 31.06; *C. vulgaris* + 15.53 ng L^{-1} IAA, 24.29; *C. vulgaris* + 44.53 ng L^{-1} IAA, 21.52; *C. vulgaris* + 51.18 ng L^{-1} IAA, 19.95.

3.4. Effect of complementation of IAA-attenuated mutant with exogenous IAA on accumulation of intra-cellular phosphate in Chlorella

In the complementation experiments, we co-immobilized *Chlorella* with the IAA-attenuated mutant *A. brasilense* FAJ0009 and supply exogenous IAA to the equivalent level of IAA produced by the wild-type *A. brasilense* Sp245. Under these conditions, accumulation of intra-cellular phosphate was even higher than when *Chlorella* was immobilized with the wild type, whether measured per culture (Fig. 4a) or per cell (Fig. 4b). For net accumulation of intra-cellular phosphate in *Chlorella*, complementation with IAA to the IAA-attenuated mutant produced phosphate levels similar to the wild type (Fig. 4c).

Analysis of acid phosphatase and alkaline phosphatase showed no effect of inoculation with either the wild type or its IAA-attenuated mutant regarding acid phosphatase (data not shown). Alkaline phosphatase was always higher in co-immobilized treatments, regardless of application of IAA (Fig. S1, supplemental material).

4. Discussion

The initial primary and critical step in acquiring phosphorus by microalgae is uptake into cells across plasma membranes. Acquisition of phosphate by microalgae, either as planktonic cells or immobilized in polymers, as was shown in this study, is essentially similar to the process in higher plants [1,2,60,61]. Two options for active/inactive proton pumps are proposed in

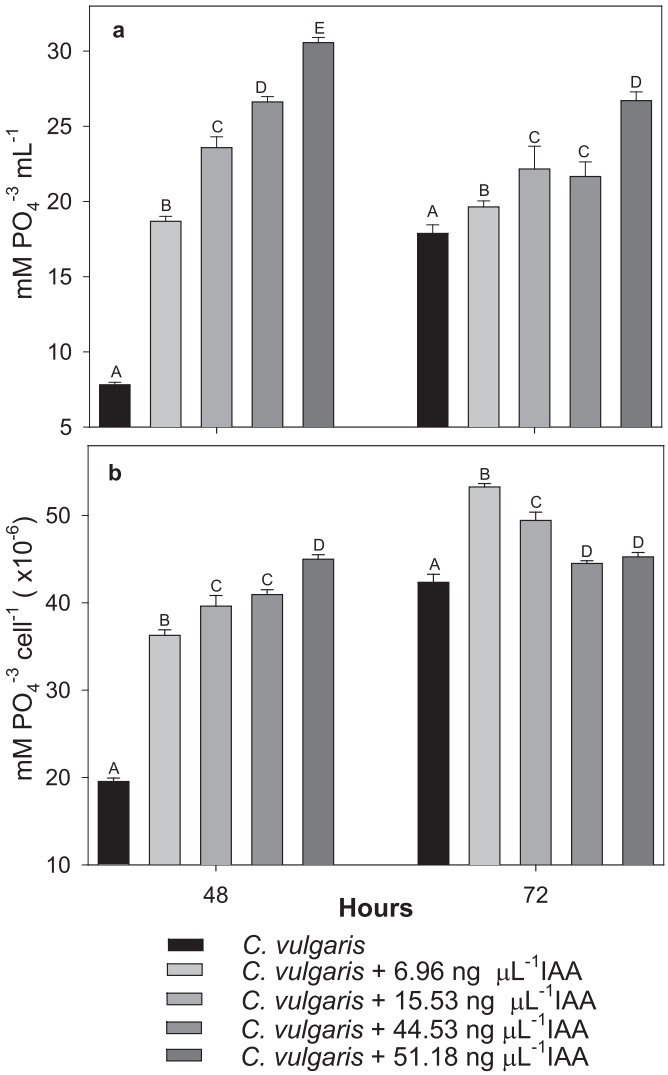

Fig. 3. Effects of different levels of exogenous IAA on accumulation of intracellular phosphate by *Chlorella vulgaris*. Accumulation of intracellular phosphate per culture (a) and per cell (b). Groups of 5 columns, in each subfigure separately denoted by different letters differ significantly by one-way ANOVA and Tukey's HSD post-hoc analysis at $p < 0.05$.

plants. (1) When a small amount of external phosphate is provided, the total amount of protons incorporated into the cytoplasm would be small because the proton pump is not activated and protons required for co-transport are provided exclusively by the medium. (2) When larger amounts of phosphate are available, as in this study, acidification of the cytoplasm occurs. As more phosphate is assimilated by the cell, more protons are absorbed. To balance across membrane potentials, the proton pump is activated, which acidifies the medium [9]. Transient activation of the proton pump by *Azospirillum* spp., sometimes yields higher absorption of phosphate. This activation was demonstrated in wheat, soybean, cowpea, and cardon cactus [62–67], but not in microalgae. The possible relation between IAA, produced in abundance by this bacteria [16,68] and phosphate metabolism has not been previously shown. This study demonstrated such a relation specifically in accumulation of intra-cellular phosphate.

Storage of important metabolites in microalgae is experimentally done in osmotically inert polymers. Inorganic phosphate is one important metabolite, and starch is the other [44,69]. Inorganic phosphate is stored within plant cells as a high molecular weight inorganic polyphosphate, as also shown in this study in *C. vulgaris*, containing a few to several hundred residues of orthophosphate that are linked by energy-rich phosphoanhydride bonds, such as in ATP [70]. This storage mechanism is found in marine and freshwater microalgae. Microalgae can accumulate more polyphosphate than is needed for immediate function of cells, [61,71,72]. The ability of microalgae to acquire phosphates in general and specifically the species used in this study is used as a biotechnological tool in tertiary wastewater treatment [50,69,73–75].

Polyphosphate plays a significant role in enhancing cell resistance under unfavorable environmental conditions [70]. Similarly, *A. brasilense* significantly improved the general performance of *C. vulgaris* [13,42] and delays senescence of microalga cells [39]. Polyphosphate is a multi-functional compound. Its most significant functions are: phosphate and energy reserves, cation sequestration and storage, membrane channel formation, participation in phosphate transport, cell envelope formation and function, control of gene activity, regulation of enzyme activities, and a role in stress response and stationary-phase adaptation. In prokaryotes, the paramount functions are energy and phosphate reserves. In eukaryotic microorganisms, regulatory functions predominate [70]. In *C. vulgaris*, this implies involvement with other regulatory functions of the cells, such as phytohormones, specifically IAA. This may happen because: (1) IAA is abundantly produced by *A. brasilense* and is well known to affect *C. vulgaris* growth [19] and metabolism [76], and (2) This study demonstrated that applying various concentrations of IAA, either synthetic or supplied by *A. brasilense* strains that produce different amounts, significantly affect polyphosphate accumulation in *C. vulgaris*. Consequently, it is plausible that a relationship, in a yet to be discovered link, includes IAA with other regulatory compounds in the cells, including polyphosphates.

IAA is thoroughly studied in higher plants [77], but little is known regarding the interaction of auxins and *Chlorella* spp. Because it is impossible to produce IAA-free *Azospirillum* mutants, studies of IAA-*Azospirilllum*-plant interaction, including interaction with microalgae, we used the IAA-attenuated mutants as the only alternative [18,19,28,29,32]. Several IAA-attenuated mutants of *A. brasilense* and *Azospirillum lipoferum* produced significantly less growth promoting effects in *C. vulgaris*, compared to their respective wild types [19]. In our study, the same mutants and wild types caused variable accumulations of intracellular polyphosphate in *C. vulgaris*.

Polyphosphate reserves in eukaryotic single-cell microorganisms are affected by phosphate availability in the medium or environmental conditions. For example, phosphate starvation of yeasts causes a quickly diminishing reserve of polyphosphates [78]. Under nitrogen starvation, the microalga *Chlorella fusca* accumulates polyphosphates [79,80], while

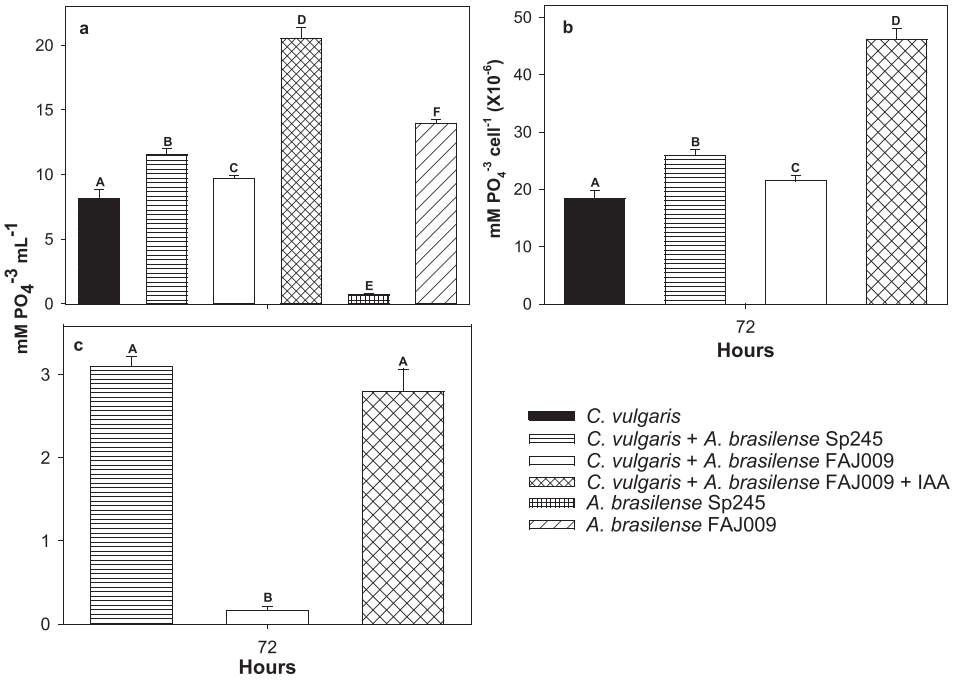

Fig. 4. The effect of augmentation of IAA-attenuated mutant *Azospirillum brasilense* FAJ0009 with exogenous IAA on accumulation of intracellular phosphate in *Chlorella vulgaris* measured per culture (a) and per cell (b). Net intracellular phosphate accumulation caused by *A. brasilense* Sp245, its IAA-attenuated mutant and complementation of the mutant with exogenous IAA (c). Columns denoted by different letters differ significantly by one-way ANOVA and Tukey's HSD post-hoc analysis at $p < 0.05$.

disassembling its photosynthesis apparatus. Polyphosphates were degraded during re-greening of microalgae [81]. Increasing temperature also increased intracellular polyphosphates [82]. In our study, with temperature held constant, phosphates were always available and this increased the amount of intra-cellular polyphosphates over time. However, when the net accumulation of cells was calculated, the accumulation over time was smaller (see Fig. 1d).

The period of interaction of the two partners in this synthetic mutualistic interaction was 72 h, where IAA was added after an initial acclimation period of 24 h. It was shown in earlier studies that, during this period, *C. vulgaris* is in its logarithmic phase of growth [13,41,44]. This period of exposure leads to optimum removal of phosphate from wastewater [50,69,75]. Therefore, longer periods of interaction under co-immobilized conditions were not included in this study.

In summary, this study shows that IAA produced by the microalgae growth-promoting bacteria *A. brasilense* was involved in regulating metabolic systems in *C. vulgaris* and led to an accumulation of intracellular polyphosphate in green microalgae.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.resmic.2015.03.001.

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