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Short communication

Alginate-perlite encapsulated *Pseudomonas putida* A (ATCC 12633) cells: Preparation, characterization and potential use as plant inoculants

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ABSTRACT

Microbial immobilization can be used to prepare encapsulated inoculants. Here, we characterize and describe the preparation of Ca-alginate-perlite microbeads loaded with cells of plant growth-promoting *Pseudomonas putida* A (ATCC 12633), for their future application as agricultural inoculants. The microbeads were prepared by dropwise addition of a $CaCl_2$-paraffin emulsion mixture to an emulsion containing alginate 2% (w/v), perlite 0.1–0.4% (w/v) and bacterial suspension in 0.9% NaCl (10^{10} CFU/mL). For all perlite concentrations used, microbead size was 90–120 μm, the trapped population was 10^{8} CFU/g microbeads and the increase in mechanical stability was proportional to perlite concentration. Microbeads containing 0.4% (w/v) perlite were able to release bacteria into the medium after 30 days of incubation. When we evaluated how *P. putida* A (ATCC 12633) entrapped in Ca-alginate-perlite (0.4% (w/v)) microbeads colonized the *Arabidopsis thaliana* rhizosphere, an increase in colonization over time was detected (from an initial 2.1×10^{4} to 9.2×10^{5} CFU/g soil after 21 days). With this treatment, growth promotion of *A. thaliana* occurred with an increase in the amount of proteins, and in root and leaf biomass. It was concluded that the microbeads could be applied as possible inoculants, since they provide protection and a controlled release of microorganisms into the rhizosphere.

1. Introduction

Numerous biotechnological processes and environmental and agricultural applications use immobilization of microorganisms in alginate beads, among other polymers, when viable microbial cells are required (Nussinovitch, 2010; Tu et al., 2015; Li et al., 2017). In particular, the encapsulation of cells for agricultural uses is performed to offer temporary protection against biotic and abiotic stress to microorganisms introduced into the soil, and to ensure a gradual and prolonged release of bacteria into the soil for the colonization of plant roots (Nussinovitch, 2010; Kim et al., 2012; Schoebitz et al., 2013; He et al., 2016).

Calcium alginate cross-linking is commonly used as an immobilization method because the procedure is simple and relatively mild, the alginate does not have any toxic effects on the cells and the matrix limits cell mortality (Young et al., 2006; Gouda, 2007; Sergio and Bustos, 2009; Ha et al., 2009; He et al., 2016; Li et al., 2017). In order to obtain alginate beads, the alginate solution (linear macromolecules comprising two monomers linked by alpha 1–4: β-acid and D-mannuronic acid to α-L-guluronic acid), is usually mixed with the cell culture and extruded into a solution of $CaCl_2$. One problem this method faces is mass transfer limitation. Reducing bead diameter has been proposed as a possible solution (Ogbonna et al., 1991). On the other hand, filling materials may be added to the alginate matrix formulation in order to improve bead mechanical resistance and cell survival inside the beads, and to allow for progressive cell release (Bashan et al., 2002; Schoebitz et al., 2013 and citations included). For instance, the use of starch in bioencapsulation reduced water content to 65% and significantly improved cell survival (Schoebitz et al., 2013). Addition of kaolin improved bead mechanical strength (Li et al., 2016). Adding chitin, bentonite or kaolin as fillers to the alginate-glycerol formulation significantly increased bacterial survival, because they provided microorganisms with protection against UVC radiation (Zohar-Perez et al., 2003). When perlite, an inorganic porous aluminosilicate with an amorphous structure, was used as an inert substrate to coat chitosan (glucosamine biopolymer) beads, adsorption capacity improved in the active sites (Kumar et al., 2010). In comparison with other inorganic supports such as silica gels, alumina, and zeolites, the addition of perlite to a polymeric matrix in order to obtain beads has advantages such as a greater mechanical stability and resistance to microbial attacks and

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organic solvents (Sarı et al., 2012).

Several *Pseudomonas* sp., such as *Pseudomonas putida* and *P. fluorescens*, have been reported as plant growth promoters (PGP) because of their metabolic properties, such as auxin or siderophore production (Kloepper et al., 1988; Saharan and Nehra, 2011). *P. putida* in particular is a species of major industrial interest within the genus *Pseudomonas* (Palleroni, 1992). In addition to its potential to degrade toxic compounds and xenobiotics, *P. putida* possesses the ability to create biofilms and colonize the plant root system, and it could be used for agricultural inoculation (Espinosa-Urgel et al., 2000; Marcus, 2003). Regarding the ability of different *Pseudomonas* strains to degrade toxic compounds, previous studies carried out by our research group showed that free-cells of *P. putida* A (ATCC 12633) were able to effectively degrade the predominant toxic cationic surfactants found in natural systems, such as tetradecyltrimethylammonium bromide and benzalkonium chloride. To make the biodegradation process more effective and improve cell tolerance to biotic and abiotic stresses, *P. putida* A (ATCC 12633) cells were immobilized in a Ca-alginate matrix. The immobilized system has advantages over free *P. putida* A (ATCC 12633) cells, since it shows greater tolerance to elevated surfactant concentrations and a greater biodegradation capacity (Liffourrena et al., 2008; Bergero and Lucchesi, 2013; Bergero et al., 2017). Taking into account the promising opportunities that *P. putida* A (ATCC 12633) offers for biotechnological uses, the aims of the present study were to obtain and characterize alginate-perlite composite microbeads containing *P. putida* A (ATCC 12633) cells and to investigate bacterial survival and release in the *Arabidopsis thaliana* rhizosphere. Our ultimate objective is to use these formulations as encapsulated inoculants to obtain long-term fertilization effects through gradual cell release.

2. Materials and methods

2.1. Bacterial strain and culture conditions

P. putida A (ATCC 12633), a collection strain of *Pseudomonas* belonging to group I (Palleroni 1992), was grown aerobically at 30 °C with shaking at 100 rpm, in Luria–Bertani (LB) medium for 8 h until reaching late exponential phase (OD_{660} 0.8–1.00; 10^{12} CFU/mL). Growth was evaluated through the reading of absorbances at 660 nm by using a visible light Beckman DU 640 spectrophotometer. To determine survival, the number of viable cells (CFU/mL) was ascertained by placing serially diluted cell suspensions on LB plates (Duque et al., 2004).

Some characteristics of *P. putida* A (ATCC 12633) related to its ability to promote plant growth were tested. Culture supernatants were biochemically tested for siderophore production on blue agar plates. Orange halos around the colonies were indicative of siderophore excretion (Schwyn and Neilands, 1987). Following Glickmann and Dessaux (1995), a culture was prepared in LB medium modified with L-tryptophan and the production of indole-3-acetic acid was quantified using Salkowski reagent. The ability of *P. putida* A (ATCC 12633) to solubilize phosphate was determined in NBRIP (National Botanical Research Institute Phosphorus) agar media. A clear area around the bacterial growth was considered a positive result (Ramachandran et al., 2007). Antagonistic activity against phytopatogenic fungi species (*Fusarium verticilloides* RC2000, *Rhizoctonia solani*, *Sclerotinia minor*, *Sclerotium rolfsii*) was tested on potato dextrose agar and trypticase-soya-agar media (McSpadden Gardener et al., 2005)

2.2. Immobilization of P. putida A (ATCC 12633) in alginate beads

P. putida A (ATCC 12633) was immobilized in calcium alginate (Ca^{2+}-alginate) in a manner similar to that described by Bergero and Lucchesi (2013), using alginic acid sodium salt from brown algae (Sigma Chemical Co., SL, USA). The final alginate concentration was 2% (w/v), bead diameter size was 2.0 mm on average, and the trapped population size reached about 1–5 × 10^6 CFU/g beads.

2.3. Immobilization of P. putida A (ATCC 12633) in Ca-alginate-perlite microbeads

To immobilize *P. putida* A (ATCC 12633) cells in a Ca-alginate-perlite matrix, microbeads were prepared via emulsification technique using expanded perlite (Perfiltra S.A., Tucumán, Argentina). For this, two emulsions were prepared. On the one hand, a mixture of Na-alginate 2% (w/v) and perlite 0.1–0.4% (w/v) (4.5 mL) was mixed with liquid paraffin (13.5 mL) and 2–3 drops of Tween 20 by stirring on a magnetic stirrer at 900 rpm for 30 min, to obtain emulsion A. About 4.5 mL of *P. putida* A (ATCC 12633) bacterium suspended in 0.9% (w/v) NaCl (approximately 10^{10} CFU/mL) were then added to emulsion A and uniformly mixed on a magnetic stirrer at 300 rpm for 20 min, to obtain emulsion B. On the other hand, 2–3 drops of Tween 20 were added to a mixture containing 0.15 M $CaCl_2$ and paraffin (2:1 (v/v)). The mixture was stirred for 30 min at 900 rpm and subsequently added dropwise with a micropipette to emulsion B under gentle stirring at $<$ 100 rpm. The alginate-perlite microbeads were collected by centrifugation at 1000x*g* for 10 min and washed three times with sterile deionized water. Later, they were filtered on a cellulose nitrate membrane (Whatman, 0.45 μm pore size) and left overnight at room temperature or at 4 °C in a refrigerator. The trapped population size reached about 10^8 CFU/g microbeads.

Diameter of the Ca-alginate-perlite microbeads was determined using a H600L microscope (Nikon, Tokyo, Japan). The average diameter was calculated from measurements of at least 30 microbeads using the image analysis program "CellA" (Olympus, Tokyo, Japan).

Microbead density was calculated using the weight and diameter of the beads, assuming that the beads were spherical. For this, the microbeads were air-dried at room temperature until constant weight was reached.

Mechanical stability was established by determination of cell release from the beads after they were stirred at 100 rpm for 24 h. The corresponding loss of entrapped cells into the medium was determined by plating serially diluted cell suspensions on LB plates (Bergero and Lucchesi, 2013).

2.4. Determination of cell release from Ca-alginate-perlite microbeads

To determine the number of cells released, 0.01 g of Ca-alginate-perlite microbeads containing about 10^8 CFU/g microbeads of *P. putida* A (ATCC 12633) cells were immersed in 10 mL of sterile physiological saline solution. At different time intervals, 100 μl samples were withdrawn under sterile conditions and centrifuged at 1000x*g* for 10 min. The supernatant was used to determine the number of viable cells (CFU/mL) by placing serially diluted cell suspensions on LB plates. The colonies were counted on agar plates after incubation for 24 h at 30 °C.

2.5. Scanning electron microscopy analysis of microbeads

For observation with scanning electron microscopy (SEM), beads were fixed following the methodology described by Bergero and Lucchesi (2013). Briefly, 0.1 g of microbeads were fixed for 5 h in 5% (v/v) formaldehyde solution in 0.1 M Tris–HCl buffer pH 7.4), washed three times in the same buffer and then dehydrated with ethanol at the following concentrations: 25% for 10 min, 50% for 30 min, 70% for 10 h and 100% for 60 min. The whole procedure was carried out at 4 °C. The samples were dried in a N_2 atmosphere. The dried samples were examined under a Zeisz scanning electron microscope at 15 kV.

2.6. Inoculation assays in Arabidopsis thaliana

A. thaliana (Col-0) seeds were surface-sterilized (5 min soaking in 70% ethanol followed by 7 min soaking in 0.5% sodium hypochlorite). They were then rinsed four times in sterile distilled water and vernalized for 2 days in the dark at 4 °C. Afterwards, the seeds were placed on

Petri dishes (10–12 seeds per plate) with Murashige and Shoog salt medium (MS) (Murashige and Skoog, 1962) containing 0.8% agar and 1% sucrose (pH 5.8). The seeds were germinated in a growth chamber (16 h light/8 h dark, 22/24 °C).

For soil experiments, seedlings (5 days old) were transplanted aseptically into pots (10 seedlings per pot) containing autoclaved soil mixture and vermiculite in a 2:1 ratio. The soil used was collected from a field in the Rio Cuarto area, Córdoba province, Argentina. The characteristics of the surface soil (< 30 cm) collected were as follows: organic content 1.47%, pH 6.80, extractable phosphorus 23.4 mg kg^{-1}soil, nitrogen of nitrate 6.3 mg kg^{-1}.

The inoculation was performed by spraying the substrate with different formulations containing the same number of viable *P. putida* A (ATCC 12633) cells: 10 mL of suspension of free-cells (10^6 CFU/mL), 10 g of Ca-alginate beads (10^6 CFU/g beads) or 0.1 g of Ca-alginate-perlite microbeads (10^8 CFU/g microbeads). Three pots containing 10 seedlings per pot were inoculated with each formulation. The seedlings were grown for 21 days in growth chamber with 16 h light/8 h dark at 22/24 °C, and were irrigated every 48 h with sterile water. Three non-inoculated pots were used as control. Experiments were repeated 3 times. Thus, for control and for each formulation, the results obtained correspond to the analysis of 30 plants per experiment.

Colonization of the soil-vermiculite rhizosphere by *P. putida* A (ATCC 12633) was determined following the methodology described by He et al. (2009). Briefly, 1 g of rhizospheric soil-vermiculite was removed from the roots and shaken in 10 mL of sterile water for 30 min. The resulting suspension was centrifuged at 1000x*g* for 10 min, and the supernatant was evaluated for CFU by plating serially diluted cell suspensions on LB plates. The plates were incubated for 24 h at 28 °C.

2.7. Measurement of A. thaliana growth parameters

For root length, main roots were measured in seedlings from the origin to the tip. The plants were dried for 5 days at 50 °C and used for protein and leaf mass determination. To determine the amount of protein, the dried plants were homogenized in 2 N NaOH and 0.1% (w/v) SDS. Following centrifugation at 10000x*g* for 30 min, the proteins were determined in the supernatant following Bradford (1976) with bovine serum albumin (Sigma Chemical Co., SL, USA) as a standard.

2.8. Statistical analysis

Analysis of variance (ANOVA) and Tukey tests were carried out using Origin (Version 8.0). All values were expressed as mean ± standard deviation (SD), differences were considered significant if $p \leq 0.05$.

3. Results and discussion

3.1. Characteristics of Ca-alginate-perlite microbeads

It is known that bacterial cells encapsulated in alginate beads and introduced into non-sterile soil survive better than cells added directly to the same soil. Because of this, for the efficient use of alginate-based inoculants it is crucial that the cells encapsulated in the beads remain viable during storage and following introduction to the soil, and are then adequately released from the beads. On the other hand, a reduction in bead size increases survival of bacteria inside and allows them to be applied by drip irrigation (Li et al., 2017 and citations included). The mechanical stability of the beads and the survival of cells in the beads are largely influenced by the properties of the support material. This material determines how the beads are able to accommodate the stress to which they are subjected in different processes. An approach to improving these parameters includes additives in the alginate-beads. Perlite is a good candidate as a filling material because of its high porosity, large surface area, abundance and low cost (Hasan et al., 2003; Hasan et al., 2006). Different concentrations of perlite (0.1%, 0.2% and 0.4% (w/v)) and a fixed concentration of Na-alginate (2% (w/v)) were used to produce Ca-alginate-perlite microbeads. The microbeads prepared were compared with respect to diameter, mechanical stability and efficiency to entrap *P. putida* A (ATCC 12633) cells (Table 1). For all perlite concentrations used, microbead diameter size ranged between 90 and 120 μm and trapped population size was constant (approximately 10^8 CFU/g microbeads). Mechanical stability increased with increasing perlite concentrations. Microbeads made without perlite did not maintain their stability over time and broke after 12 h, with a corresponding loss of cells into the medium. In the field of bacterial inoculation technology, one of the benefits presented by the encapsulation of microorganisms is that beads decrease their weight, which is a function of density. Density values measured for beads with increasing perlite concentration are shown in Table 1. We detected that microbead density decreased with increasing perlite concentration. Thus, although microbeads made with both 0.2% and 0.4% (w/v) perlite had good mechanical stability and did not display any structural changes or damage after being stirred for 24 h, beads obtained with 0.4% (w/v) perlite showed lower density and could therefore be chosen over the others to formulate inoculants.

Table 1
Physical properties and cell viability of *P. putida* A (ATCC 12633) microbeads prepared with 2% (w/v) of Na-alginate solution and different concentrations of perlite.

Perlite concentration (% w/v)	0	0.1	0.2	0.4
Diameter size (μm)	101 ± 12	105 ± 10	106 ± 11	109 ± 15
Density (g/mL)	1.18 ± 0.3	1.08 ± 0.6	1.02 ± 0.5	0.95 ± 0.4
Stability	Not stable	Not stable	Stable	Stable
Entrappedcells	4.5×10^8	7.1×10^8	5.2×10^8	5.5×10^8
Viability (CFU/g beads)				
4 °C	3×10^8	6×10^8	5×10^8	4×10^8
24 °C	6×10^8	5×10^8	3×10^8	7×10^8

Values are means ± SD (n = 3).

On the other hand, an important goal of industrial formulations with immobilized microbial cells is to maintain the cells entrapped in an active form for as long as possible (Schoebitz et al., 2013). Different types of media are used for storing beads because the type of storage solution may decrease microcapsule stability (Lee et al., 2010). From day one, we used as storage solutions both 0.9% (w/v) NaCl and a buffered medium (20 mM HCl-Tris pH 7.4, 44 mM KCl, 85 mM NaCl and 0.8 mM $MgSO_4$) that does not contain phosphate, since this ion is known to cause extensive disruption in Ca-alginate beads (Sossa-Urrego et al., 2008; Bergero and Lucchesi 2013). Although for 150 days we did not observe any visual disintegration of the Ca-alginate-perlite microbeads in either solution, there was low cell leakage from the matrix when microbeads were maintained in the buffered medium, likely due to the presence of non-cross linking divalent ions such as Mg^{+2} (De Vos et al., 2009). In order to evaluate the effect of temperature on storage conditions, microbeads were suspended in a 0.9% (w/v) NaCl solution and kept refrigerated (at 4 °C) or at room temperature (24 °C). As can be seen in Table 1, the initial number of entrapped bacteria was similar to the number of cells contained within the beads after incubation, for beads stored at 24 °C and 4 °C (approximately 10^6 CFU/mL). This means that microbeads can be stored at both temperatures for at least 150 days with no appreciable loss of viability.

3.2. Cell release from Ca-alginate-perlite microbeads

Ca-alginate microbeads with or without perlite containing *P. putida* A (ATCC 12633) cells were tested for their ability to release entrapped bacteria for a total of 30 days. Fig. 1 shows the CFU cumulative release of cells (i.e. the amount of living cells released from the microbeads,

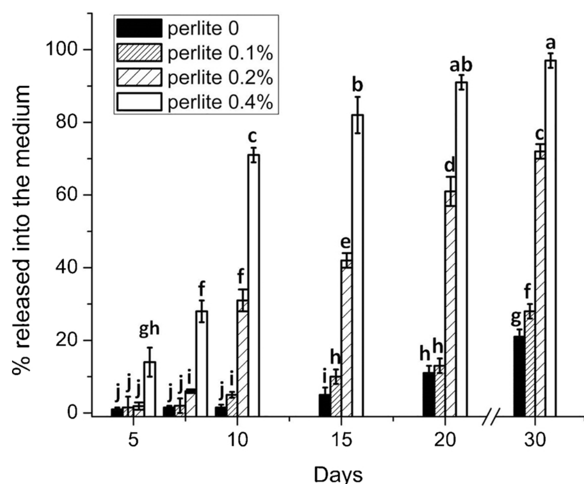

Fig. 1. *P. putida* A (ATCC 12633) cell release from Ca-alginate-perlite microbeads. The microbeads were prepared with Ca-alginate 2% (w/v), varying perlite concentration from 0 to 0.4% (w/v). 0.0.1 g of Ca-alginate-perlite microbeads containing about 10^8 CFU/g microbeads of *P. putida* cells were immersed in 10 mL sterile physiological saline solution at room temperature to determine the cells released by plate count.
Data presented are mean ± SD from three replicates of each experiment. Values with different letters in superscript indicate significant differences at P 0.05.

expressed as a percentage of the total amount of cells incorporated into the microbeads), measured at each point in time. Microbeads containing 0.2 and 0.4% (w/v) perlite were able to release bacteria into the medium, while microbeads without or with 0.1% (w/v) perlite did not release significant numbers of bacteria into the medium, even after 30 days of incubation. As shown in Fig. 1, the release of cells from microbeads containing 0.2 and 0.4% (w/v) perlite can be divided into two phases. In the first phase, corresponding to the first 7 days, the rate of bacteria released from the capsules was the slowest. After this time, for both types of beads, cells were more rapidly released and accumulated in the medium. After 15 days of incubation, the amount of cells released was higher for microbeads containing 0.4% (w/v) perlite than for those containing 0.2% (w/v) (approximately 8.2×10^5 CFU/mL (80%) and 4.2×10^5 CFU/mL (40%), respectively). This indicates that modifying the perlite concentration in the matrix formulation of Ca-alginate microbeads can lead to greater cell release. The increase in cell release observed in microbeads with perlite could be attributed to the fact that when perlite interacts with alginate, a less compact structure is formed. As shown in Fig. 2, alginate microbeads prepared with perlite showed a

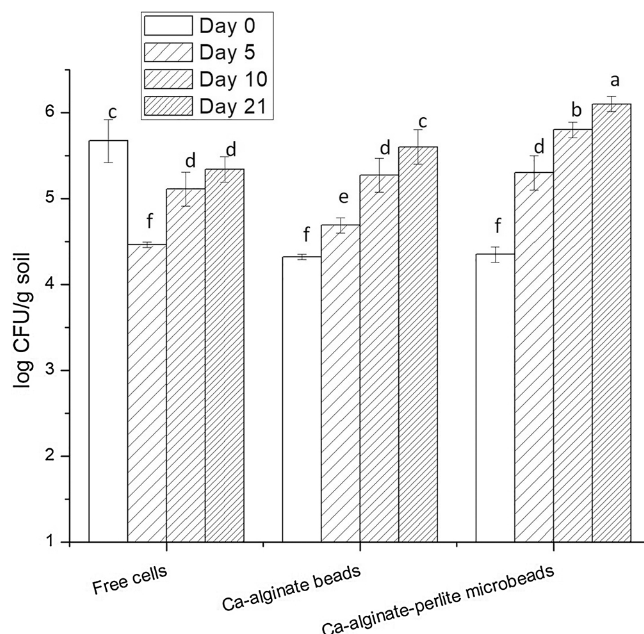

Fig. 3. Colonization of *A. thaliana* rhizosphere by *P. putida* A (ATCC 12633) in various formulations: free cells, Ca-alginate beads and Ca-alginate-perlite microbeads. 5-day-old seedlings were inoculated with 10^6 CFU/mL of *P. putida* free-cell suspension, 10 g of Ca-alginate beads (10^6 CFU/g beads) or 0.1 g of Ca-alginate-perlite microbeads (10^8 CFU/g microbeads).
Data presented are mean ± SD from three replicates of each treatment. Values with different letters in superscript indicate significant differences at P0.05.

less compact surface structure with increased surface roughness and porosity, characteristics which are known to decrease mass transfer resistance (Lu et al., 2012). Thus, porosity increased and more channels were formed in the beads containing perlite, resulting in greater bacterial release.

3.3. Colonization and capacity to promote the growth of *A. thaliana* by *P. putida* A (ATCC 12633) cells

With the purpose of evaluating which would be the best formulation for its future application as an agricultural inoculant, we compared the ability of *P. putida* A (ATCC 12633) cells to colonize the *A. thaliana* rhizosphere when applied in different formulations: as free cells, entrapped in alginate beads, or in Ca-alginate-perlite microbeads (perlite 0.4% (w/v)). As shown in Fig. 3, after 5 days a large reduction in bacterial number was observed when non-encapsulated cells were used

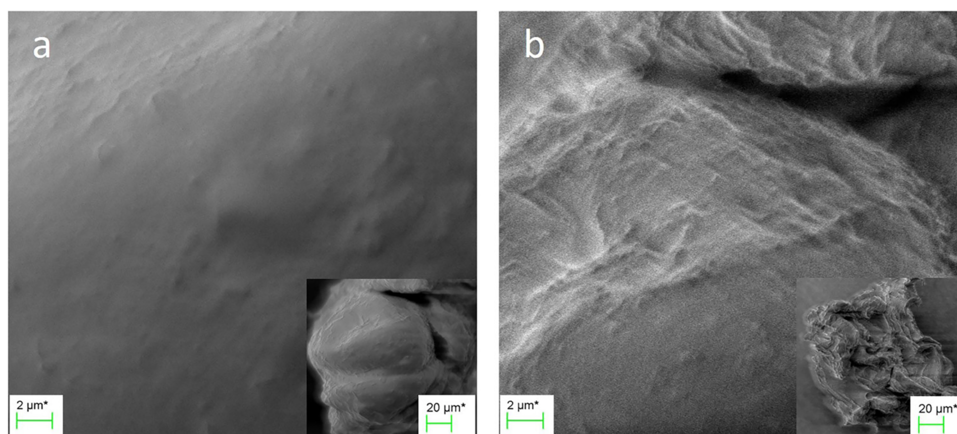

Fig. 2. Scanning electron micrographs (SEM) of the whole microbead and the surface of the bead. a) Ca-alginate microbead, b) Ca-alginate-perlite microbead (perlite 0.4%).

Table 2
Growth promotion of *A. thaliana* inoculated with different formulations containing *P. putida* A (ATCC 12633) cells.

Formulations	Root length (cm)	Leaf mass (mg/plant)	Amount of protein (μg/mg fresh weight)
Control	8.12 ± 1.41[a]	9.03 ± 1.25[a]	24.93 ± 3.25[a]
Free cells	13.94 ± 1.21[b]	13.38 ± 1.42[b]	34.80 ± 1.86[b]
Ca-alginate beads	10.56 ± 1.11[c]	11.53 ± 1.65[b]	31.52 ± 2.18[b]
Ca-alginate-perlite microbeads	15.52 ± 2.12[b]	17.51 ± 1.32[d]	38.38 ± 3.12[c]

Inoculation was performed with different formulations containing the same number of viable *P. putida* A (ATCC 12633) cells: 10 mL of free-cells (10^6 CFU/mL), 10 g of Ca alginate beads (10^6 CFU/g beads) or 0.1 g of Ca-alginate-perlite microbeads (10^8 CFU/g microbeads). Control: without inoculation. Data presented are mean ± SD from three replicates of each treatment. Values with different letters in superscript indicate significant differences at P 0.05.

to colonize the rhizosphere (from 4.7×10^5 CFU/g dry soil to 2.9×10^4 CFU/g dry soil after 5 days). Although the cells detected in the rhizosphere increased in number 10 and 21 days after their application as free cells, it was not possible to recover the initial number of bacteria applied. The highest colonization occurred when the *A. thaliana* rhizosphere was treated with Ca-alginate-perlite microbeads. As shown in Fig. 3, an increase in colonization over time was detected. At 21 days, the number of living cells per g of soil in the rhizosphere was similar to the inoculated amount (from 2.1×10^4 CFU/g soil to 9.2×10^5 CFU/g soil after 21 days). These results are related to the characteristics of Ca-alginate-perlite microbeads and their ability to release entrapped cells over time (Fig. 1). In addition, this behavior demonstrates once again that immobilization has more advantages for microorganisms than free-living suspensions. These advantages include protection against environmental stress and probably a supply of nutrients without competition from other microorganisms. Also, the colonization of *A. thaliana* by *P. putida* A (ATCC 12633) cells obtained with Ca-alginate-perlite-microbeads was greater than that obtained with Ca-alginate-beads (Fig. 3). This can be attributed, in part, to the smaller size of the alginate-beads that containing perlite (109 μm and 2 mm with and without perlite, respectively). It has been reported that smaller beads are less fragile than larger beads and have the advantage of a higher surface to volume ratio, allowing for good maintenance of the trapped cells (Robitaille et al., 1999; Poncelet and Neufeld, 1989). In addition, the reduction in bead diameter has been proposed as a possible solution to mass transfer limitation (Ogbonna et al., 1991). Diffusion limitations within larger-sized beads can reduce cellular metabolism, and the lack of supply of essential substances to the interior of the beads may lead to cell death. In addition, despite the small size (109 μm), Ca-alginate microbeads without perlite as filling material could not be used as inoculants because, as shown in Table 1, they did not maintain their stability over time and broke after 12 h with liberation of trapped cells.

As was described previously (Materials and Methods section), the results of laboratory studies showed that *P. putida* A (ATCC 12633) produces indole-3-acetic acid, excretes siderophores, solubilizes phosphates and has antagonistic activity against different phytopatogenic fungi species. Consequently, *P. putida* A (ATCC 12633) has the capacity to promote plant growth. As shown in Table 2, for *A. thaliana* seedlings inoculated with free cells, the increase in the amount of protein 28 days after inoculation was 42%, in comparison with non-inoculated plants. The increase in root and leaf biomass was 72 and 45% respectively. However, as with the colonization of the rhizosphere, the best results concerning growth promotion of *A. thaliana* were obtained when the treatment was performed with Ca-alginate-perlite microbeads. Regarding non-inoculated plants, the increase in the amount of protein, root and leaf biomass was 54, 93 and 94%, respectively (Table 2). Although it is not shown, the empty microbeads (without microorganisms) did not improve plant growth when compared to non-inoculated controls. Therefore, the findings of the present work demonstrate that Ca-alginate-perlite-microbeads containing *P. putida* A (ATCC 12633) cells are the best alternative for the formulation of inoculants to improve growth in *A. thaliana*.

In summary, this study describes a simple method to produce alginate-perlite microbeads containing bacterial cells, which can serve as inoculants through the gradual release of cells with the capacity to promote plant growth.

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