Heliyon 6 (2020) e05218

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Phosphate-solubilizing *Pseudomonas* sp., and *Serratia* sp., co-culture for *Allium cepa* L. growth promotion

Andrea Blanco-Vargas [a,b,c,1], Lina M. Rodríguez-Gacha [a,1], Natalia Sánchez-Castro [a,1], Rafael Garzón-Jaramillo [a], Lucas D. Pedroza-Camacho [a], Raúl A. Poutou-Piñales [b], Claudia M. Rivera-Hoyos [b], Lucía A. Díaz-Ariza [c], Aura M. Pedroza-Rodríguez [a,*]

[a] *Laboratorio de Microbiología Ambiental y de Suelos, Unidad de Investigaciones Agropecuarias (UNIDIA), Departamento de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, D.C., Colombia*

[b] *Laboratorio de Biotecnología Molecular, Grupo de Biotecnología Ambiental e Industrial (GBAI), Departamento de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, D.C., Colombia*

[c] *Laboratorio Asociaciones Suelo, Planta Microorganismos (LAMIC), Grupo de Investigación en Agricultura Biológica, Departamento de Biología, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, D.C., Colombia*

ARTICLE INFO

Keywords:
Biofertilizer
Phosphate rock
Pseudomonas sp.
Serratia sp.
Allium cepa
Crop production
Field crops
Organic farming
Bacteria
Microbial biotechnology
Biotechnology
Plant biology
Agriculture

ABSTRACT

Different genus of bacteria has been reported with the capacity to solubilize phosphorus from phosphate rock (PR). *Pseudomonas* sp., (A18) and *Serratia* sp., (C7) isolated from soils at the "*Departamento de Boyacá*" Colombia, where *Allium cepa* is cultivated. Bacteria were cultured in MT11B media and evaluated as a bio-fertilizer for *A. cepa* germination and growth during two months at greenhouse scale. *Pseudomonas* sp., and *Serratia* sp., cultured at 30 °C, 48 h in SMRS1 agar modified with PR, (as an inorganic source of phosphorus), presented a phosphate solubilization index (SI) of 2.1 ± 0.2 and 2.0 ± 0.3 mm, respectively. During interaction assays no inhibition halos were observed, demonstrating there was no antagonism between them. In MT11B media growth curve (12 h) demonstrated that co-culture can grow in the presence of PR and glucose concentrations 7.5-fold, lower than in SMRS1 media and brewer's yeast hydrolysate; producing phosphatase enzymes with a volumetric activity of 1.3 ± 0.03 PU at 6 h of culture and 0.8 ± 0.04 PU at 12 h. Moreover, co-culture released soluble phosphorus at a rate of 58.1 ± 0.28 mg L^{-1} at 8 h and 88.1 ± 0.32 mg L^{-1} at 12 h. After five days of evaluation it was observed that germination percentage was greater than 90 % of total evaluated seeds, when placing them in contact with the co-culture in a concentration of 1 × 10^8 CFU mL^{-1}. Furthermore, it was demonstrated that co-culture application (10 mL per experimental unit to complete 160 mL in two months) at 8.0 Log_{10} CFU mL^{-1} twice a week for two months increased *A. cepa* total dry weight (69 ± 13 mg) compared with total dry weight (38 ± 5.0 mg) obtained with the control with water.

1. Introduction

Indiscriminate and excessive chemical fertilizer use has an adverse effect on physical, chemical and biological properties of the soil, deteriorating the soil's quality and in time diminishing agricultural productivity [1, 2, 3]. Therefore, a need to evaluate alternative mineral and/or organic mineral fertilizer sources arises. These alternatives must support nutritional crop requirements, favor high yield and quality production that comply with agricultural sector sustainability indices. In this manner the soil's biological processes should not be affected, and the quality of hydric resources should not be altered, since high nutrient release induces hypertrophication [4].

Phosphate rock (PR) is a natural source of inorganic phosphorous that can be an alternative to chemically synthesized fertilizers. Its rational use, employed by itself or in combination with other organic products would help diminish the effect of intensive agricultural practices [1, 2, 3]. Colombia is a privileged country, since it has important PR deposits, located in the Departments of Boyacá, Huila, Norte de Santander and Cauca. In PR, phosphorous pentoxide (P_2O_5) content varies between 20 and 30 % (w/v), [5]; however, despite its high phosphorous content it is

* Corresponding author.
E-mail address: apedroza@javeriana.edu.co (A.M. Pedroza-Rodríguez).
[1] These authors contributed equally to this work and should be considered first author.

https://doi.org/10.1016/j.heliyon.2020.e05218
Received 28 June 2019; Received in revised form 21 May 2020; Accepted 8 October 2020

found as an un exchangeable form with low solubility at neutral and alkaline pH [6]. Therefore, it is not an available phosphorus source for plants, requiring chemical or microbiological pre-treatment to favor insoluble nutrient transfer, promoting phosphorous solubilization and plant absorption [7, 8].

Combined use of PR and phosphorous solubilizing bacteria (PSB) in different vegetables crops, such as bulb onion (*A. cepa*), could help increase P source and availability for the plant's metabolism at seedbed and food plot, since P is required for seed germination, early root growth and bulb thickening [9, 10].

Phosphorous solubilizing bacteria (PSB) represent 20–40 % of the soil's microbiota characterized by their metabolic versatility, capability of colonizing roots and diverse enzyme and metabolite production [11, 12]. In addition, they have the capacity to solubilize phosphate minerals, such as di- and tri-calcium phosphate, hydroxyapatite and PR [10, 13, 14]. Different bacteria genera have been reported capable of solubilizing phosphorus, such as *Pseudomonas* spp., *Bacillus* spp., *Rhizobium* spp., *Burkholderia* spp., *Achromobacter* spp., *Agrobacterium* spp., *Micrococcus* spp., *Aerobacter* spp., *Flavobacterium* spp., *Mesorhizobium* spp., *Azotobacter* spp., *Azospirillum* spp, *Erwinia* spp., and *Serratia* spp., [15, 16, 17, 18, 19, 20, 21, 22].

To obtain a biological product required stages include understanding the mechanisms of P solubilization, bacteria selection, design and election of culture media and production conditions for a PSB based bioinoculate. PSB culture can use different organic and inorganic phosphorus sources, such as PR, tri-calcium phosphate, agroindustry byproducts, rich in nitrogen and organic phosphorus [14, 23]. Employing other phosphorus sources, PSB can produce metabolites, such as enzymes, protons, siderophores, among others that aid in P solubilization [17].

On the other hand, bacteria must be supplied of a carbon source, where they can obtain energy to produce organic acids that actively participate in P solubilization [24, 25, 26, 27, 28]. This element could be found as a precipitate or fixed with other elements, mainly depending on pH and soil type. In acid soils, free hydroxyls and Al^{+3} and Fe^{+3} hydroxyls fix P, whereas in alkaline soils P is fixed by Ca^{+2} and Mg^{+2} [29, 30].

The objective of this work was to evaluate *Pseudomonas* sp., and *Serratia* sp., potential to solubilize P from PR, grown in the alternate culture media MT11B, and its evaluation as a bio-inoculant on *A. cepa* growth at greenhouse scale.

2. Materials and methods

2.1. Microorganisms and molecular identification

Gram-negative A18 and C7G isolates were obtained from soils where *A. cepa* was cultured in the "*Departamento de Boyacá, Colombia*" (Sample Colletion. Strain A18: Punta Larga 5º47′03.5″N and 72°58′52.6″W and strain C7G: Pesca 5°36′58.5″N and 73°01′42.0″W). This procedure was carried-out at the Laboratorio de Microbiología Ambiental y de Suelos, Pontificia Universidad Javeriana. These strains were reactivated from a primary cell bank (PCB) [31] stored at -20 °C in BHI broth supplemented with 25% (w/v) glycerol, which was reactivated in Brain Heart Infusion agar (BHI) incubated at 30 °C for 24 h. Molecular identification was performed by sequencing a 1,465 bp region corresponding to the 16S rRNA, using 337F, 518F, 907R and 110R primers (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4044206/). Subsequently, sequences were manually curated and assembled to obtain consensus sequences. Taxonomic analysis was performed comparing consensus sequences against data from *NCBI* (*National Center for Biotechnology Information,* https://www.ncbi.nlm.nih.gov), and *RDP* (*Ribosomal Database Project,* http://rdp.cme.msu.edu). Sequences available from RefSeq data base [32] with the highest homology were used to perform a multiple alignment with Clustal W [33]. Phylogenetic trees were obtained by maximum likelihood methods based on the Jukes-Cantor [34] model with 1,000 replica Bootstrap using MEGA X software [35].

2.2. Solubilization index and soluble phosphorus concentration

Suspensions for each bacterium were prepared in 0.85 % (w/v) saline solution at a 9.0 Log_{10} CFU mL^{-1} and 9 microdrops were seeded out of 20 μL in SMRS1 agar modified with PR pH 7.2 ± 0.2. PR recipe was 5.0 g L^{-1} PR (Calboy®; http://www.calboy.co, 2018) (25 % P_2O_5, 32 % CaO, 14 % SiO_2, 0.5 % Al_2O_3 (w/w), 10 g L^{-1} glucose, 0.5 g L^{-1} yeast extract, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.2 g L^{-1} KCl, 0.3 g L^{-1} $MgSO_4$, 0.004 g L^{-1} $MnSO_4$, 0.0004 g L^{-1} $FeSO_4$, 0.2 g L^{-1} NaCl, and 20 g L^{-1} agar. Petri dishes were cultured at 30 °C for 48 h. Solubilization index (SI) was determined employing Eq. (1) [23].

$$SI = \frac{A}{B} \quad (1)$$

where: A is the colony halo in mm + solubilization halo diameter in mm, B colony diameter (mm). Results correspond to average of three replicas ± SD.

For preliminary solubilization and growth assays modified SMRS1 broth with PR was used, which had the same composition as the media previously described, except without agar. One hundred milliliter Erlenmeyer flasks were used with 25 mL media inoculated with 5 % (v/v) of a co-culture inoculum (1:1, *Pseudomonas* sp., + *Serratia* sp.). Erlenmeyer flaks with contents were cultured for 72 h at 30 °C and 120 rpm. Biomass production was determined by decimal dilution technique (Log_{10} from CFU mL^{-1}) and count in Petri dish containing modified SMRS1 agar. Ortho-phosphate concentration (mg L^{-1}) was determined using Spectroquant® phosphate reagent test (MQuantTM phosphate test, Merck-Millipore), [36]. Each determination was performed in triplicates.

2.3. Gauze technique for interaction determination

Antagonism assays between both bacteria were performed using the agar diffusion technique. Each strain was reactivated in BHI agar and 10 × 10^8 cell mL^{-1} suspensions were prepared in 0.85 % saline solution (w/v). Then, 0.1 mL of one strain of bacteria suspension was seeded on the surface with a Drigalsky spatula. Subsequently, three filter paper disks impregnated with 0.1 mL of the bacterial culture of the other bacterial strain to be confronted at a concentration of 10 × 10^8 cells mL^{-1} were placed on top of the agar for interaction determination. For negative control filter paper disk impregnated with dH_2O was used. Petri dishes were incubated at 30 °C for 48 h. Antagonism was determined by massive presence or absence of zone of inhibition on the seeded bacteria on the agar. Results were expressed in mm [23].

2.4. Growth curves

Growth curves were obtained along 12 h cultures in two different media. Media composition were: SMRS1 broth (5 g L^{-1} tricalcium phosphate, 10 g L^{-1} glucose, 0.5 g L^{-1} yeast extract, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.2 g L^{-1} KCl, 0.3 g L^{-1} $MgSO_4$, 0.004 g L^{-1} $MnSO_4$, 0.0004 g L^{-1} $FeSO_4$ and 0.2 g L^{-1} NaCl, pH 7.2 ± 0.2) and MT11B broth (5.0 g L^{-1} PR, 2.5 g L^{-1}glucose, 0.5 g L^{-1} brewer's yeast hydrolysate, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.2 g L^{-1} KCl, 0.3 g L^{-1} $MgSO_4$, 0.004 g L^{-1} $MnSO_4$ $7H_2O$, 0.0004 g L^{-1} $FeSO_4$ and 0.2 g L^{-1}NaCl, pH 7.2 ± 0.2).

All components in MT11B media are employed in food industry and production of inorganic fertilizers. In addition, brewer's yeast hydrolysate is a byproduct of Colombian beer industry. Growth curves were performed in triplicate in 100 mL Erlenmeyer flasks with 25 mL work effective volume (WEV), 10 % inoculum (v/v), 200 rpm at 30 °C. Samples were collected every 2 h and response variables were SMRS1 agar colony count (CFU mL^{-1}) [23], residual glucose (g L^{-1}) by 3,5-dinitrosalicylic acid assay (DNS) [37], pH and *o*-phosphate concentration (mg L^{-1})

[36], determined using Spectroquant® phosphate reagent test (MQuantTM three phosphate test, Merck). All determinations were performed in triplicate.

Colony count (CFU mL^{-1} expressed as Log_{10} CFU mL^{-1}), were performed in modified SMRS1 with 5.0 g L^{-1} PR. For orthophosphate concentration determination, cultures were centrifuged for 20 min at 3,578 x *g*. Supernatant was collected to determine *o*-phosphate concentration using Spectroquant® phosphate reagent test (MQuantTM three phosphate test, Merck). Absorbance read at λ_{357} nm was used to calculated *o*-phosphates concentration by using a standard curve (0.5–6.0 mg L^{-1} *o*-phosphates with the following equation y = 0.1657x – 0.008, R^2 = 0.9970).

Moreover, phosphorus yield in glucose (Equation 2), biomass (measured as CFU mL^{-1}) volumetric productivity (Equation 3), phosphatase activity and *o*-phosphate (Equation 4) productivity were calculated. To determine possible productivity and yield significant differences, a comparison of means (mean ± SD) between culture media was made ($\alpha = 0.05$). Additionally, with results from each media a multiple correlation analysis was performed (Pearson). All tests were carried-out with SAS® software for Windows (SAS Institute 2017. version STAT 9.0. Cary, NC: SAS Institute) with a 95 % confidence interval.

$$Y(p/s) = \frac{Pf - Pi}{So - Sf} \quad (2)$$

where: $Y_{(p/s)}$ is the product yield (phosphorus in solution) divided by the substrate (glucose), P_f is a final product (soluble phosphorus released from the solubilization of the PR) concentration, Pi: initial product concentration, S_0 initial substrate concentration, S_f final substrate concentration.

$$P(x) = \frac{Xf - Xo}{T} \quad (3)$$

where: $P_{(x)}$ is the biomass productivity, X_f is final biomass concentration, X_0 is initial biomass concentration, T is time where the maximum biomass concentration was obtained

$$P(p) = \frac{Pf - Po}{T} \quad (4)$$

where: $P_{(p)}$ is the product productivity P_f is final product, P_0 is initial product, T is time where the greatest amount of product expressed as phosphatase or phosphorus in solution was observed, respectively.

2.5. Determination of phosphatase activity

For phosphatase activity determination protocol described by Angulo-Cortés *et al.*, (2012) was used with one modification. Briefly, samples collected at 0, 4, 6, 8 and 12 h were centrifuged at 3,578 x *g* for 20 min at 19 °C. 200 μL of collected supernatant for each time point was placed into a sterile tube to which 255 μL 0.1 M 4-*p*- nitrophenyl phosphate prepared in universal MUB buffer at pH 7.0 ± 0.2 was added. Solution was incubated for 1 h at 37 °C. To stop the reaction 360 μL of 0.5 M NaOH and 86 μL of 0.5 M $CaCl_2$ were added. Tube was centrifuged at 3,578 x *g* for 10 min at 19 °C. Absorbance was read at OD_{400} nm in Genesis-20 spectrophotometer. All samples were in triplicate. To establish concentration a *p*-nitrophenol standard curve was prepared (0.01–0.06 μmol mL^{-1}, equation: y = 13.53x + 0,0024, R^2 = 0.9960). For this assay one phosphatase activity (1 PU) is equal to one *p*-nitrophenol μmoL min^{-1} L^{-1} released under reaction conditions [23].

2.6. HPLC high resolution liquid chromatography

To identify produced organic acids in MT11B culture media, 20 μL culture supernatant were collected and injected into a chromatograph for HPLC analysis. A SH1011 column with 0.01N H_2SO_4 mobile phase was used. Flow was kept at 0.6 mL min^{-1}, at 35 °C. Detected organic acids were identified at their retention time and the area under the curve (AUC) was compared to known standards with a 210 nm UV detector [7, 26, 38].

2.7. Bacterial co-culture effect on Allium cepa seedling growth

These experiments were performed in two phases. The first phase was associated with seed germination percentage at the laboratory. The second phase corresponded to seedbed establishment. For this assay *A. cepa* Granex standard bulb seed were used. To determine germination percentage 20 *A. cepa* seeds were placed in triplicate into a 9.0 cm diameter Petri dish, containing a Whatman No. 3 filter paper. To each Petri dish 5 mL of co-culture suspension was added at a concentration of 8.0 Log_{10} CFU mL^{-1}. As a control dH_2O was used and MT11B sterile media was diluted 1/1000 to achieve application dose [39]. For each strain, co-culture and control Petri dishes were incubated for 5 days at 20 ± 2 °C in the dark; time at which, the number of germinated seeds was determined by radicle sprouting (3 mm) from the testa [40]. Germination percentage was then calculated [41]. Collected data was used to determine mean ± SD significant differences between controls, each individual strain and co-culture. SAS statistical analysis software SAS® (SAS Institute 2017. version STAT 9.0. Cary, NC: SAS Institute) was used to determine significant differences.

For seedbed establishment at greenhouse level, agricultural soil was employed as substrate collected from plots where *A. cepa* is grown in Boyacá. Soil was sterilized in two 15-minute autoclave cycles at 1.2 atm and 121 °C with a 24 h interval between cycles. Additionally, the soil was irradiated with UV light at 254 nm for 12 h, using a cubic photolysis reactor [42]. For seed planting 266 mL plastic cups containing 100 g soil were used. In each cup seven *A. cepa* seeds were placed 3 cm deep covered with soil, which received different treatments: 10 mL co-culture (8.0 Log_{10} CFU mL^{-1}), 10 mL each strain (8.0 Log_{10} CFU mL^{-1}) and 10 mL sterile dH_2O. Each treatment was performed in triplicate.

Experimental units were watered every fourth day for a period of two months with the products previously described, for a total of 16 applications equivalent to 160 mL of product and/or water or culture medium in two months of evaluation. Response variables were seedling emergence percentage (the seven seeds sown in the ground, for each replica were 100 %, the number of seeds that emerged to become a seedling was determined; with this value, seed emergence percentage was calculated) to leaf height in cm (measured from the base of the leaf to the inflorescence apex), dry weight in mg (each plant was dried at 85 °C for 48 h) [41], total PSB count and PSB count per morphotype using modified SMRS1. Last, comparison between mean ± SD for all treatments: control, each individual strain and co-culture was determined to establish significant differences among treatments. SAS statistical analysis software SAS® (SAS Institute 2017. version STAT 9.0. Cary, NC: SAS Institute) was used to determine significant differences.

3. Results

3.1. Molecular identification

According to NCBI RefSeq/16S ribosomal rRNA results A18 strain (927 bp consensus sequence) was classified within the genus Pseudomonas with 99 % homology in 100 % of its entire sequence in comparison with *Pseudomonas koreensis* and *Pseudomonas moraviensi*. However, in the phylogenetic analysis greater closeness with *Pseudomonas koreensis* was observed, suggesting it could belong to this species (Figure 1A). On the other hand, taxonomic analysis compared to NCBI RefSeq/16 S ribosomal rRNA for the C7 strain 842 bp assembled sequence, classified the strain within the Serratia genus with 99 % homology and 100 % of its entire sequence in comparison with sequences belonging to *Serratia liquefaciens*, *Serratia quinivorans* and *Serratia grimesii*. These results were in

agreement with phylogenetic analysis, where this strain was not particularly grouped with any species (Figure 1B). Considering C7 strain demonstrated a high phosphate solubilization index, it could be identified as *Serratia liquiefaciencs*, since it has been reported this species has great capability to solubilize inorganic phosphate [30].

3.2. Solubilization index and interaction assays

Both bacteria solubilized phosphorus from PR, obtaining a SI of 2.1 ± 0.2 and 2.0 ± 0.3 mm for *Pseudomonas* sp., (A18) and *Serratia* sp., (C7), respectively. After 48 h of incubation under experimental conditions no inhibition halos were observed, demonstrating at interaction assays that no antagonism was observed between both bacterial strains. Therefore, a positive or neutral interaction could be established that would allow for co-culture production. For preliminary growth and solubilization assays in SMRS1 supplemented with 5 g L^{-1} PR, soluble phosphorus concentrations for each individual bacteria strain were 66.2 ± 13.4 and 89.5 ± 4.7 mg L^{-1}, with colony counts of 9.0 ± 0.5 and 8.3 ± 0.9 Log units at 72 h for *Pseudomonas* sp., and *Serratia* sp., respectively (Table 1). At present study, no proportional correlation between SI and PS concentration (mg L^{-1}) was observed.

3.3. Growth curves and production

Design and condition selection for MT11B media were previously performed by our group (data not shown). Thus, this improved media was evaluated for co-culture production and was compared to frequently reported in the literature SMRS1 conventional media [23, 43]. As illustrated in Figure 2A *Pseudomonas* sp., or co-culture did not show an adaptation phase. For both cultures, exponential growth phase finished at 8 h, with Log_{10} CFU mL^{-1} values of 10.4 ± 0.009 for co-culture and 10.1 ± 0.029 for *Pseudomonas* sp. On the other hand, for *Serratia* sp., the exponential phase was maintained up to 4 h, followed by a stationary phase up to 10 h of culture. The highest counts for these bacteria were 10.2 ± 0.023 Log_{10} CFU mL^{-1} at 8 h of culture. Regarding to growth by morphotype, it was determined that *Serratia* sp., colony proportion was approximately 0.18 times greater than *Pseudomonas* sp., colonies, which could be associated with greater growth rate and affinity for carbon and/or nitrogen source. Nevertheless, this difference did not support *Serratia* sp., was inhibiting *Pseudomonas* sp., growth, since colony count remained above 8.0 logarithmic units during and at the end of the growth curves (Figure 2A). In regard to SMRS1 production media colony count results were similar in comparison to MT11B media (Figure 2B). No adaptation phase was observed, and exponential growth finished at 8 h with 10.1 ± 0.08, 10.0 ± 0.02 and 10.3 ± 0.04 Log_{10} CFU mL^{-1} for co-culture, *Pseudomonas* sp., and *Serratia* sp., respectively (Figure 2B).

In MT11B, initial glucose concentration was 2.5 g L^{-1} with a pH of 7.2 ± 0.2 for co-culture. Residual glucose ended at 0.3 g L^{-1} and a pH of 3.6 ± 0.2 at 12 h; suggesting that pH, decrease could be due to organic acid production, resulting from glucose aerobic metabolism (Figure 2A). In SMRS1, initial glucose concentration was 7.6 ± 0.1 g L^{-1} with a pH of 7.2 ± 0.2. At 12 h of culture residual glucose was 1.01 ± 0.01 g L^{-1}. The final pH SMRS1 was 4.1 ± 0.6; higher compared to MT11B (Figure 2B).

HPLC analysis for organic acids detected the production of gluconic acid (845.5 mg L^{-1}), oxalic acid (3.6 mg L^{-1}), citric acid (14.2 mg L^{-1}) and malic acid (11.8 mg L^{-1}) at 8 h of co-cultivation. The production of gluconic acid and the release of soluble P in the medium were associated.

The highest soluble phosphate (SP) concentration released in MT11B was 88.1 ± 0.32 mg L^{-1}, at 12 h, with a positive correlation with biomass production up to 8 h of culture, since both variables increased (ρ = 0.96,

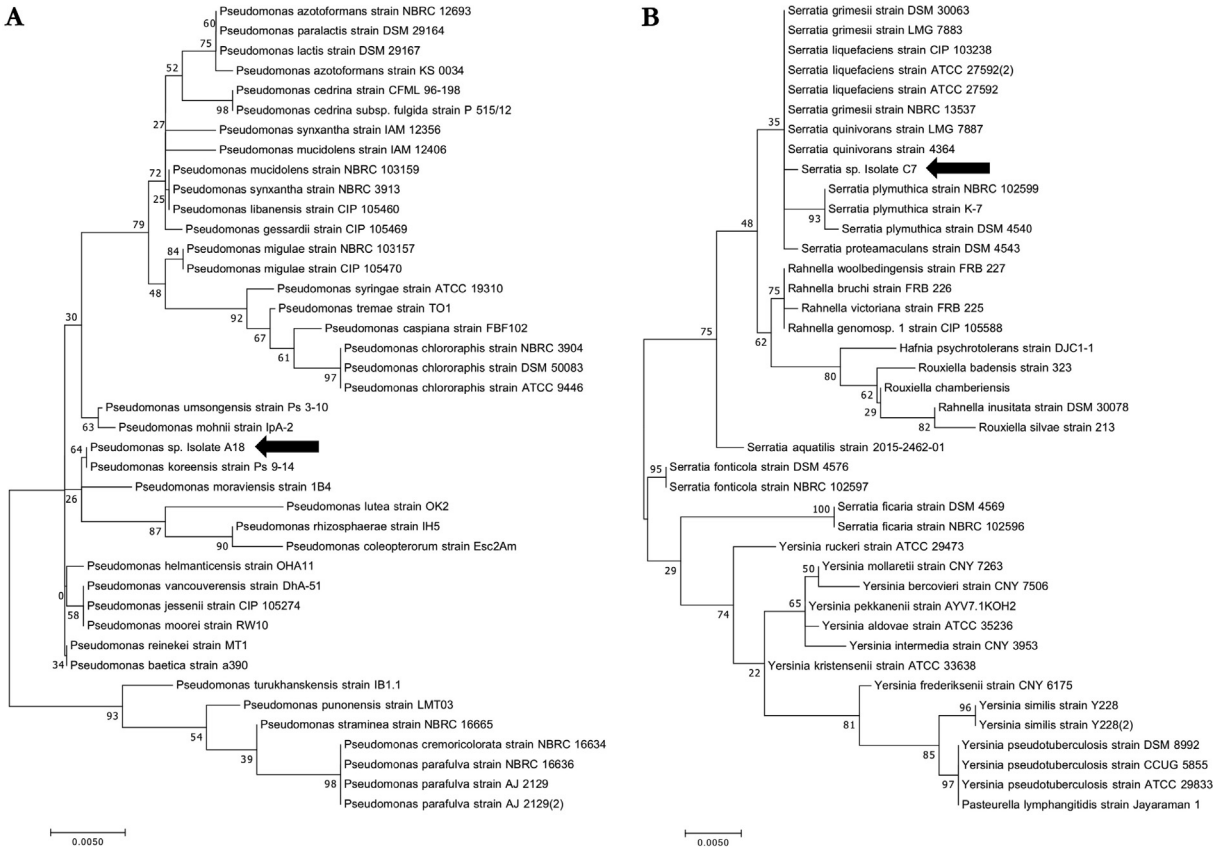

Figure 1. *Pseudomonas* spp., (A18) and *Serratia* spp., (C7) 16S rRNA phylogenetic analysis. (A) Evolutionary history using *Maximum Likelihood* method based on Jukes-Cantor model with *Bootstrap* of 1,000 replicas for *Pseudomonas* spp. (B) Evolutionary history using *Maximum Likelihood* method based on Jukes-Cantor model with *Bootstrap* of 1,000 replicas for *Serratia* spp. Percentage trees grouped with associated taxa are illustrated next to the branches. Trees are presented at scale, with branch length according to the number of substitutions per site. Analysis for each case included 41 NT sequences. A18 (A) and C7 (B) isolates are indicated with black arrow.

Table 1. Preliminary solubilization results and Gauze interactions.

Strain	Solubilization Index	Inhibition halos (mm)	Soluble phosphorus (mg L^{-1})	Log_{10} of CFU mL^{-1}
Pseudomonas sp.	2.1 ± 0.2	0 ± 0	66.2 ± 13.4	9.0 ± 0.5
Serratia sp.	2.0 ± 0.3	0 ± 0	89.5 ± 4.7	8.3 ± 0.9

Average of three replicas (mean ± SD). Coefficient variation less than 20%.

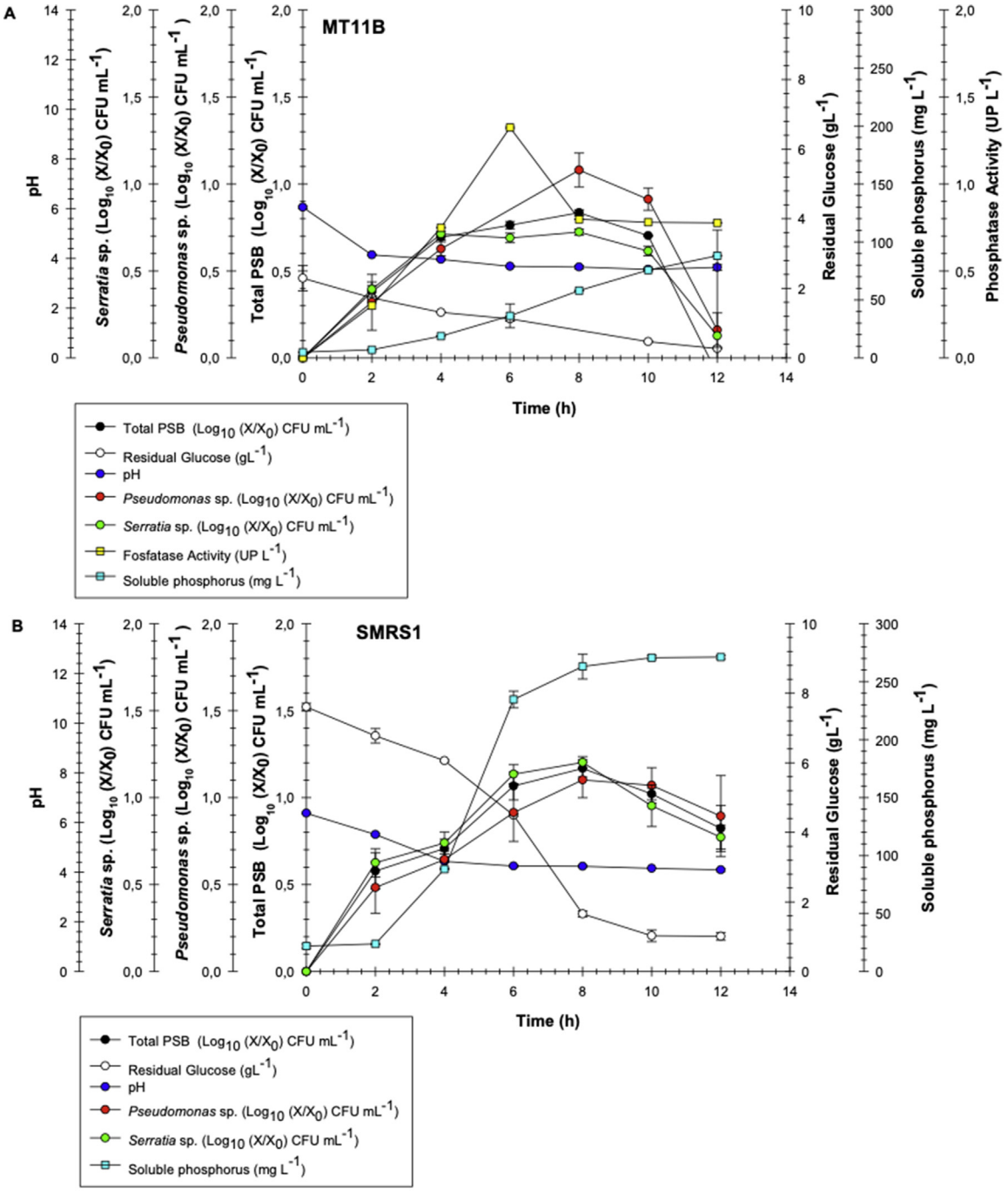

Figure 2. Growth curve for 12 h co-culture (A) MT11B media at 30 °C and 200 rpm. (B) SMRS1 media at 30 °C and 200 rpm. Results are average of three replicas ± SD.

$p < 0.0012$). In contrast, SP was negatively correlated with pH ($\rho = -0.90$, $p < 0.0010$) and residual glucose ($\rho = -0.90$, $p < 0.0017$), because PSB can increase P availability through different processes. These include inorganic P solubilization and mineralization of organic P, using various mechanisms and combinations thereof, such as decreasing pH, organic acid release and proton extrusion from PR (Figure 2A).

For the SMRS1 media, SP concentration at 12 h of culture was greater in comparison with MT11B media (SMRS1: 271.2 ± 1.3 mg L^{-1}).

Table 2. Co-culture kinetic parameters in MT11B and SMRS1 media at 8 h of evaluation.

Parameter	MT11B at 8 h	SMRS1 at 8 h
$P_{(biomass)}$ (CFU mL^{-1} h^{-1})	$3 \times 10^9 \pm 7 \times 10^{7a}$	$4 \times 10^9 \pm 3 \times 10^{8a}$
Phosphatase activity* PU	1.3 ± 0.04	ND
$P_{(phosphatase)}$* (U L^{-1} h^{-1})	0.21 ± 0.01	ND
$P_{(Soluble\ P\ (SP))}$ (mg L^{-1} h^{-1})	7.3 ± 0.03^b	32.9 ± 1.3^a
$Y_{(Soluble\ phosphorus/glucose)}$ mg mg^{-1}	**$0.065 \pm 0.03^{a,**}$**	0.044 ± 0.002^b
$Y_{(Soluble\ phosphorus/CFU)}$ mg CFU^{-1}	$2 \times 10^{-12} \pm 5 \times 10^{-14b}$	$4 \times 10^{-2} \pm 2 \times 10^{-3a}$

a, b are related with significant differences obtained after ANOVA test.
* Activity and phosphatase activity were determined at 6 h of culture.
** Result in bold was significantly different.

Phosphorus concentration in solution positively correlated with colony count ($\rho = 0.98$, $p < 0.0004$) and negatively with residual glucose and pH ($\rho = -0.94$, $p < 0.0016$) (Figure 2B).

Phosphatase activity was only quantified in MT11B media, where a gradual increase was observed until reaching a maximum volumetric activity of 1.3 ± 0.03 UP at 6 h of culture, with a gradual decrease to end with an activity of 0.8 ± 0.04 UP at 12 h (Figure 2A). Each bacterial strain presented alkaline phosphatase activity of 2.9 ± 0.1 and 0.1 ± 0.08 UP for *Pseudomonas* sp., and 2.2 ± 0.08 and 0.6 ± 0.09 UP for *Serratia* sp., at 6 and 12 h, respectively. Although, phosphatase activity in co-culture was low it correlated positively with colony count ($\rho = 0.89$, $p < 0.0096$) up to 6 h of culture, possibly favored by the source of organic phosphorus provided by the brewer's yeast hydrolysate from which mineralization processes can be carried out.

Once it was determined for both media that at 8 h of culture the highest colony counts were obtained, a comparison of means between the results of productivity and biomass yields between both media was performed (mean ± SD) (Table 2). For colony count, expressed as CFU mL^{-1} h^{-1} no significant differences were observed between MT11B and SMRS1 ($p > 0.056$). Volumetric productivity based on phosphatase activity in MT11B media was 0.21 ± 0.01 U L^{-1} h^{-1}. Moreover, SP was significantly higher in SMRS1 (32.9 ± 1.3 mg L^{-1} h^{-1}, $p < 0.0079$) in comparison with MT11B media (7.3 ± 0.03 mg L^{-1} h^{-1}).

Significant differences were observed for soluble phosphorus yield with respect to consumed glucose concentration with a higher yield in MT11B media, with a P value/mg of consumed glucose of 0.065 ± 0.03 mg mg^{-1} ($p = 0.0018$) in comparison with SMRS1 media 0.044 ± 0.002 mg mg^{-1}. Based on 8 h of production results, namely colony count and SP yield, MT11B media can become an alternative for SMRS1 broth for PSB *Pseudomonas* sp. and *Serratia* sp., co-culture (Table 2). This media contains low cost components and agroindustry byproducts (brewer's yeast hydrolysate as a source of nitrogen and organic phosphorus).

3.4. Allium cepa seed germination percentage

Significant differences were observed for germination percentage in wet chamber for individual strains, co-culture and controls with MT11B sterile media ($p < 0.0001$). Observed percentages were 53 % for *Serratia* sp., and 63 % for *Pseudomonas* sp. Strain co-culture resulted in higher germination percentage (91 %). Last, for controls germination percentages were 79 % for dH_2O and 83 % for MT11B at 1/1000 dilution. These results showed employing co-culture at 8.0 Log_{10} CFU mL^{-1} favored *A. cepa* seed germination. Additionally, results demonstrated neither bacteria, nor MT11B media components exerted an adverse effect on germination (Figure 2).

3.5. Greenhouse assays employing agricultural soil

An acid not saline clay loam soil was employed, with low content of organic matter and a C/N ratio of 12 (Table 3). In addition, this soil had high available phosphorus concentrations (250 mg kg^{-1}) and medium concentrations of aluminum and iron; suggesting that, at pH 4.72 ± 0.2

Table 3. Nutrient and physico-chemical characterization of agricultural soil used for greenhouse evaluations.

Parameter	Value	Unit	Analytical methos
Clay-loam texture pH	4.7		[44] Saturated paste/pH meter
Electric conductivity	1.06	(dS m^{-1})	Saturated extract/conductimeter meter
Apparent density	1.03	g cc^{-1}	Calculated
Organic carbon	3.99	(%)	Colorimetric [45]
Organic matter	6.87	(%)	Calculated
Total nitrogen	0.32	(%)	Micro-Kjeldhal [46]
C/N ratio	12		Calculated
Exchangeable potassium	289	mg kg^{-1}	Ac. NH_4/atomic absorption
Exchangeable sodium	60	mg kg^{-1}	Ac. NH_4/atomic absorption
Iron	42	mg kg^{-1}	Acid mix/atomic absorption/Melhich I [47]
Boron	0.49	mg kg^{-1}	$Ca(OH)PO_4$/Colorimetric
Aluminum	17	mg kg^{-1}	Calculated
Phosphorus	250	mg kg^{-1}	Bray II solution [48]/Colorimetric
Ammonium	39	mg kg^{-1}	NaCl/Colorimetric
Nitrates	58	mg kg^{-1}	Ac. Na/Colorimetric
Total bacterial count	5.4	UFC g^{-1}	Petri dish colony count
PSB count	2.9	UFC g^{-1}	Petri dish colony count
Phosphatase activity	ND	UP L^{-1}	*p*-nitrophenyl phosphate [49]/Colorimetric

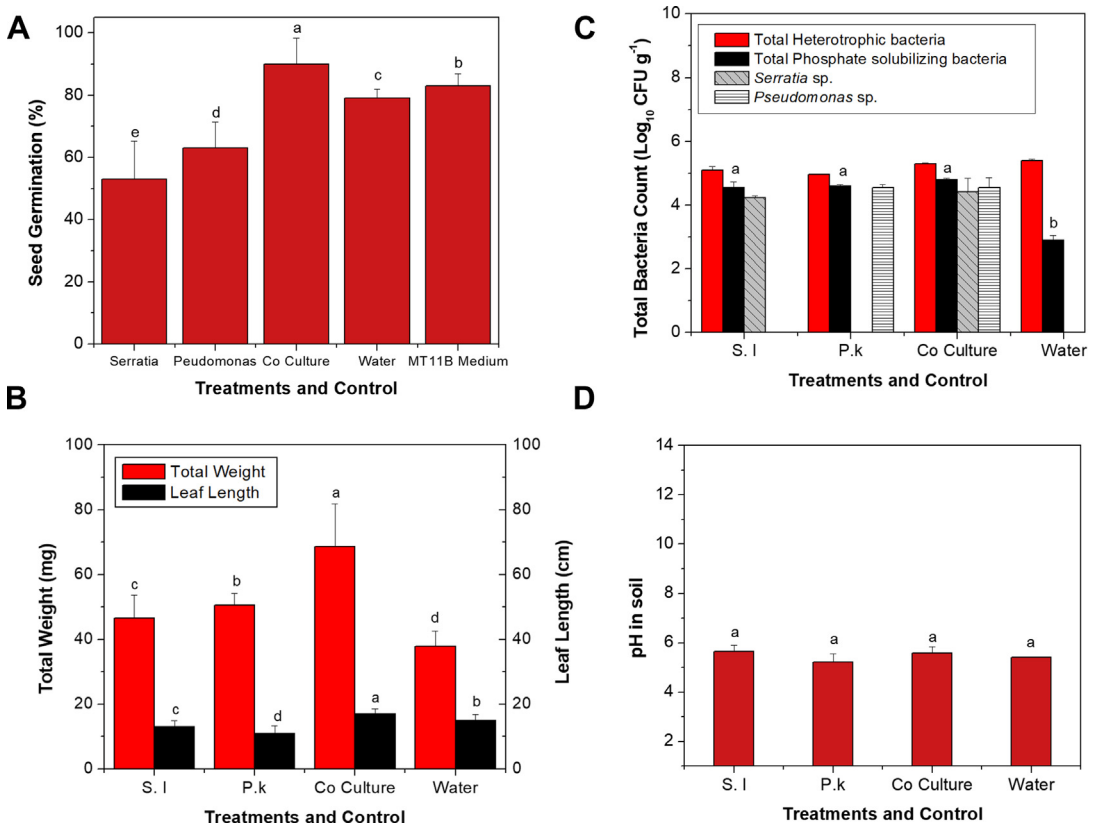

Figure 3. (A) *A. cepa* seed germination percentage. (B) Total dry weight and leaf length (C) Total bacterial count (D) Soil's pH. a, b, c, d and e letters above bars in figures (A), (B) y (C) indicate presence of heterogeneous groups, hence significant differences.

phosphorus could be as phosphate ion and aluminum phosphate. In regard to cultured microorganisms in the soil sample, a total of 5.4 logarithmic units of total bacteria were observed, possibly associated with organic matter, since soils with low content of organic matter tend to contain low microorganism concentrations. Total PSB counts were low (2.9 logarithmic units) and no phosphatase activity was detected in the soil.

In relation to the seed emergence percentage in soil, significant differences were observed for the co-culture with respect to the control with distilled water ($p < 0.0001$), obtaining 82, 55 and 48 %, for the co-culture, *Pseudomonas* sp., and *Serratia* sp., respectively. In the control with distilled water, the emergence percentage was 75 %. The percentages were lower than those obtained in the experiments with a humid chamber, even so, it is confirmed that the application of the two bacteria in co-culture again favors the emergence of the seeds in the soil, exceeding the control with water.

For total dry weight significant differences among co-culture, individual bacterial strains and control with dH_2O were observed when agricultural soil was employed for greenhouse assays (Figure 3B). Thus, demonstrating co-culture application at 8.0 Log_{10} CFU mL^{-1} twice a week for two months favoured increase in (69 ± 13 mg ($p = 0.0001$). In the individual application of the isolates, the total dry weight for *Pseudomonas* sp., was 50 ± 4 mg ($p = 0.0023$) and for Serratia sp., it was 47 ± 7 mg ($p = 0.0030$). Last, for control *A. cepa* total dry weight 38 ± 5.0 mg ($p = 0.0078$). The leaf height inoculated with co-culture was 17.3 ± 0.8 cm, followed by plants watered with *Pseudomonas* sp. (13.8 ± 0.2 cm) and *Serratia* sp. (11.7 ± 0.5 cm). Last, seedlings watered with dH_2O for two months had an average leaf height of 15.4 ± 1.9 cm (Figure 3B).

Total heterotrophic bacteria count in microbiological analyses in soil samples showed counts were similar and ranged between entre 5.0 ± 1.0 and 5.4 ± 1.1 logarithmic units ($p > 0.0001$). However, total PSB count were significantly higher in treatments (co-culture, *Pseudomonas* sp. or *Serratia* sp.) in comparison with control ($p < 0.0001$) (Figure 3C). Furthermore, results demonstrated bacteria were stable in soils during evaluation period, since under greenhouse conditions the concentration of liquid products initially applied was 8.0 Log_{10} CFU mL^{-1}, and decreased to 5.0 Log_{10} CFU g^{-1}.

Morphotype count in co-culture and for each individual strain retained an approximate proportion of 4.4 ± 0.7 and 4.6 ± 0.4 logarithmic units for *Serratia* sp., and *Pseudomonas* sp., respectively. These values were very similar, demonstrating bacteria remained viable in the soil. Our data demonstrate a desirable treatment effect when developing a co-culture under greenhouse evaluation.

pH slightly increased for all treatments (initial pH was 4.72 ± 0.2), reaching values of 5.6, 5.2, 5.6 and 5.4 ± 0.2 for co-culture, *Pseudomonas* sp., *Serratia* sp. and control with dH2O without any significant difference ($p > 0.01$) at two months (Figure 3D).

Last, positive correlations were observed for the co-culture experiments under greenhouse conditions for dry weight ($\rho = 0.91$, $p < 0.0033$), seedling height ($\rho = 0.92$, $p < 0.0067$ and PSB count ($\rho = 0.91$, $p < 0.0055$). Therefore, demonstrating *Serratia* sp. and *Pseudomonas* sp. PSB are directly associated with seed germination and plant growth. In addition, they favored nutrient availability, such as phosphorus, originating from organic acid and hydrogen ion production, in addition to alkaline phosphatase activity. These are metabolites produced from a carbon source, or by the mineralization of compounds containing organic phosphorus.

4. Discussion

4.1. Bacteria characterization

Bacteria such as *Pseudomonas* spp., and *Serratia* spp., can be isolated from soils where different plants are cultivated, such as grass (*Miscanthus*

sinensis, Lolium perenne), maize (*Zea mays*), vanilla (*Vanilla planifolia*) and onion (*Allium cepa*), [8, 50, 51, 52, 53, 54]. Little is known about *Allium cepa* crop, with few reports in the literature regarding isolated bacteria from soils, where onions have been cultivated. Furthermore, not much is known about bacterial effect on phosphorus solubilization and availability of this element for growth and development of this vegetable.

Different authors have reported both genera have the capacity to solubilize inorganic phosphorus (tricalcium phosphate and PR), mineralize organic phosphorus (phosphatase activity), produce substances promoters of vegetable growth and substances for biological control of rhizosphere phytopathogens [16, 17, 52, 55, 56]. When co-cultured they can increase and complement their potential as a bio-inoculant, in such manner the plant can benefit during different stages of development, such as seed germination, seedling propagation, seedling in seedbeds and cultivation in plots. In this manner a product suitable for commercialization and at lower cost and greater benefits for the soils and plant compared with 100% chemical fertilization can be obtained [3, 51, 55]. Henceforth, the interest arises to characterize two bacteria isolated from subtropical soils and evaluate their possible use as bio-inoculants in *A. cepa* crops.

4.2. Solubilization index and interactions

To develop a co-culture biofertilizer it is indispensable for each bacterial strain to maintain its biological activity, during bio-inoculant production or during its application at seedbed level, greenhouse or plot, without generating an antagonistic effect against the other strain [23, 57, 58]. This was proven by SI estimation, during preliminary solubilization assays in liquid media and during interaction evaluation in solid media.

Both bacterial strains solubilized PR in solid as well as liquid media at a concentration of 5 g L^{-1}. This was supported by a decrease in pH, since organic acid production, such as gluconic, oxalic, citric and malic acid dissociate PR and release ortho-phosphates (Table 1), [4, 16, 17]. These acids are produced during carbohydrate metabolism, such as glucose, fructose, saccharose, and maltose among others [7, 24, 25].

Differences between solubilization halos and SP concentrations might be due to variations between bacteria on the type of acid produced, chemical structure, molecular weight, or diffusion velocity [7, 19]; even though all acids produced by bacteria are water soluble and are excreted outside of the cell. Various authors have reported similar results in trying to correlate the results of both techniques, and agree bacteria selection must not only be based on SI, considering other criteria must be taken into account, such as concentration of the soluble element, biomass quantity and produced organic acid concentration [16, 17, 21, 23, 43, 59, 60].

Solubilization index and SP concentration when cultured in PR for *Pseudomonas* sp. and *Serratia* sp., were similar to those reported in the literature, where tricalcium phosphate was used as the source of phosphorus [17, 25, 52]. Thus, demonstrating despite being the less soluble source of phosphorus, it was feasible to solubilize phosphorus and obtain high biomass concentrations [8, 25, 53]. HPLC analyses revealed that co-culture in MT11B media produced gluconic, oxalic, citric and malic acid. Behera *et al.*, (2017) reported presence of various organic acids, such as malic, lactic and acetic acid by *Serratia* sp., [17]. Alam *et al.* (2002) reported citric, oxalic, acetic and gluconic acid in phosphate solubilizing microorganism's culture broth [61]. Glucose is one of the main components of MT11B media, and PSB have preference for glucose as a carbon source to produce organic acids, which can increase P availability in soil [26].

Under evaluated experimental conditions in this work Gauze interaction assays demonstrated neither bacteria produced diffusible substances to the media that would inhibit the other strain. In addition, amensalism by antibiotic production, hydrolytic enzymes or bacteriocins were not observed in co-culture [62]. Consequently, a favourable result was observed and both strains can be formulated in co-culture or applied in co-inoculation and possibly during bio-inoculant production and further use at greenhouse level, maintaining positive or neutral interaction [63].

Colony count was another result supporting both strain's potential as possible bio-inoculants. Values were high (8.0 and 9.5 Log_{10} CFU mL^{-1}) at 72 h of discontinuous culture in comparison with other reports, where tricalcium phosphate or PR were used as P sources, and CFU did not exceed 7.0 Log_{10} CFU mL^{-1} [24,52,64]. When obtaining elevated biomass concentrations, the bio-inoculant could be dosed, in such as way in the field it would be used at concentrations ranging between 3.0 and 4.0 logarithmic units, guaranteeing the establishment of bacteria in the soil, since competition with the microorganisms found in the soil are high, and even heavy metals could inhibit their growth [52, 65].

4.3. Growth curves and production

Various aspects must be considered when producing complete culture media including viable cells and their metabolites (organic acids, hydrogen ions, growth promoter substances and phosphatases) for two-fold purpose bio-inoculant formulation [4, 7, 66]. Among the factors considered are media composition associated with sources of carbon, nitrogen, phosphorus, raw material costs and time of processes. Therefore, it was necessary to evaluate alternative media different from SMR1, Pikovskaya and NBRIP (*National Botanical Research Institute's phosphate*) that would give the same or better yield and productivity in shorter production time and with reasonable costs in comparison with traditional SMR1 media.

Growth by morphotype was also evaluated, where only for *Pseudomonas* sp. a lower growth was observed in the first hours of the process (6 h). Nevertheless, subsequently count equaled growth with respect to co-culture and *Serratia* sp. These results could be associated with *Pseudomonas* sp. reduced capacity to solubilize phosphorus. In preliminary assays *Pseudomonas* sp. produced 66.2 mg L^{-1} phosphorus in comparison with *Serratia* sp. with 89.5 mg L^{-1} (Table 1).

Colony counts were similar to those reported by other authors [23], who used PR and *Saccharomyces cerevisiae* hydrolysate with a 24 h production time. Hence, demonstrating similar results can be obtained with MT11B media, reducing production time by 16 h. Additionally, counts in MT11B media exceeded some results reported in traditional media for PSB culture. Ludueña *et al.* (2017) obtained 9.0 logarithmic units after 20 h in NBRIP media with tricalcium phosphate [64], one logarithmic unit less than for MT11B media with 12 h more hours of culture. Chen *et al.* (2006) cultured for 3 days Gram-negative bacteria in mineral media supplemented with 5.0 g L^{-1} tricalcium phosphate and obtained *Serratia marcescens* counts between 8.7 and 9.3 logarithmic units [67].

Carbon, nitrogen and phosphorus sources must be provided in both media to favour bacteria co-culture growth. As it has been reported in the literature, bacteria of the genera Pseudomonas and Serratia can employ different sources of carbon and nitrogen (organic and inorganic) and phosphorus (tricalcium phosphate, hydroxyapatite, iron phosphate, aluminum phosphate and PR [7, 16, 66]. However, they have higher affinity for hexoses such as glucose, which can be metabolized by the oxidative pathway and its intermediate metabolites, enter the Krebs cycle and then the electron transport chain and oxidative phosphorylation for energy production [7, 16, 68]. Omar (1998) reported PR can increase biomass growth, when used at concentrations greater than 0.5 g per 100 mL, demonstrating inorganic phosphorus is not only raw material for solubilization, but is also used for structural compound production and precursors for the production of energy [57].

Additionally, when analyzing biomass productivity results (CFU mL^{-1} h^{-1}) for both media no significant differences were observed ($p > 0.056$). Thus, demonstrating co-culture can grow in MT11B media in presence of PR, brewer's yeast hydrolysate (agroindustry byproduct) and glucose concentrations at 7.5 times lower than in SMRS1 media. For this media (SMRS1) final glucose concentration was 1.0 g L^{-1}, a higher value in comparison with MT11B media (0.3 g L^{-1}). These results suggested co-

culture consumes more efficiently glucose when initial concentration is > 10 g L^{-1}.

Under this condition glucose consumption velocity was 0.612 h^{-1}, a higher value in comparison with SMRS1media (0.501 h^{-1}), which is an important saving in the direct costs of production in terms of quantity used, raw material and process time (8 h) costs. When performing raw material cost analysis for both culture media it was calculated 1 L of MT11B was 0.03 USD, 52 times less in comparison with SMRS1 media ($ 1.6 USD/L), becoming a production alternative for this type of bacteria.

As carbon source was consumed, pH in media decreased to 3.6 and 4.1 ± 0.2 for MT11B and SMRS1 media, respectively. It is known inorganic phosphorus solubilization is due to different mechanisms, such as organic acid release, proton extrusion by ammonium assimilation and carbonic acid production from microbial respiration [69]. In this study pH decrease was mainly attributed to gluconic, oxalic and malic acid production from glucose metabolism and organic nitrogen metabolism [70]. From the above mentioned, it has been reported the most effective process for phosphorus solubilization by Gram-negative bacteria is gluconic acid production. This acid is produced by direct oxidation of glucose mediated by glucose dehydrogenase (E.C. 1.1.1.49) and the presence of pyrroloquinoline quinone (PQQ) cofactor [16, 68]. Once gluconic acid is produced it can be oxidized to an intermediate compound 2-ketogluconic acid. Both products are strong carboxylic acids that can release ions to the mineral's surface by anionic exchange or by cation chelation bonded to phosphate groups [7, 24, 57].

Another compound that could have favored pH decrease was brewer's yeast hydrolysate. It is considered that organic sources of nitrogen can simultaneously supply carbon and nitrogen, promoting in short times elevated biomass concentration, unlike inorganic nitrogen sources [14]. Additionally, during nitrogen mineralization it can produce ammonium, and its assimilation favours proton excretion (H^+), contributing to decreased pH [7].

Phosphate solubilization from inorganic sources varies depending on the initial source, where tricalcium phosphate, hydroxyapatite, iron phosphate and aluminum phosphate and PR, are the most used to study solubilization mechanisms [7, 16]. In the present study PR was extracted from the "*Departamento de Boyacá*", Colombia. Phosphoric rock chemical characterization determined it was composed of various elements, where phosphorus was not the principal element: carbon (9%), oxygen (53.24%), aluminum (0.57%), silicon (4.48%), phosphorus (11.18 %) and calcium (21. 42%). Absence of flour (F) guaranteed the culture was not inhibited by this element, since it has been reported its presence in PR decreases solubilization, which is directly associated with loss of viability in phosphosolubilizing bacteria and fungi [59].

Phosphate rock characterization results indicated it had a moderate P concentration, which was not in free form, because it was for the most part bound to iron, and to a lesser extent to aluminum. Henceforth, to release orthophosphate ions into the media bacteria were required. These microorganisms can grow under this form of phosphorus, and release P in a soluble form into the media. Phosphate solubilization was measured by free orthophosphates in the media, as in centrifuged samples biomass and sediment solids were separated.

Soluble phosphate release from PR must be carried-out under acid conditions, which was achieved in this work by co-culture with bacterial strains that used carbon and nitrogen sources resulting in a decrease in pH. Furthermore, organic acid solubilization was a process that could be partly associated with growth, since SP was observed from the beginning, but continued during the stationary phase and even into the death phase. These events could be associated with growth decrease, since part of the dead cells could have released intracellular components with acid characteristics, which were not necessarily organic acids produced from glucose metabolism. Therefore, various authors report high solubilization efficiencies require prolonged processes, more so than for biomass production. Paul *et al.* (2018) suggested maximum solubilization values can be obtained between 72 and 96 h [56]. A similar result was reported by Sreenivasulu *et al.*, (2014). In their work they demonstrated SVUNM17 strain isolated from mines in Moscow, solubilized phosphate rock with 76.10 mg L^{-1} *o*-phosphate production after 28 days in culture [71].

Indeed, orthophosphate solubilization takes longer than biomass production. Therefore, when developing a bio-inoculant for seedbed and plot use, it is more favourable to obtain a product with elevated biomass concentrations, even though *o*-phosphate concentration is not as high. Basically, because at a higher cell concentration it is more feasible bacteria continue their solubilizing activity in the soil, and rapidly colonize the rhizosphere. Although available phosphorus can be immobilized by cations present in the soil, and not remain available to the plants, when the product is applied [72, 73, 74].

On the other hand, co-culture use or consortia guarantee greater solubilization efficiency, since more than one bacteria strain use can produce different types of organic acids simultaneously. In addition, other solubilization mechanisms can be activated and eventually bacteria could produce substances that promote plant growth. Lara-Mantilla *et al.*, (2015) and Nandimath *et al.,* (2017) demonstrated bacteria consortia use at 10×10^8 CFU mL^{-1} compared with individual strains and in co-culture increases phosphorus solubilization [75, 76].

Organic phosphorus minealization must have taken place by extracellular phosphatase production. Brewer's yeast hydrolysate may have contributed to some extent to organic nitrogen and phosphorus. Parhamfar (2016) demonstrated when evaluating phytate by itself or mixed with tricalcium phosphate it favoured microorganism growth and phosphatase production [14]. Our results are in agreed with those reported by Parhamfar (2016), since MT11B media contained a mix of organic and inorganic phosphorus. Organic phosphorus might have in part come from phosphatase activity on P in brewer's yeast hydrolysate. Increasing brewer's yeast hydrolysate might be associated with higher concentrations of organic phosphorus. Finally, co-culture is a bio-inculant with multipurpose, because it presents elevated biomass concentration, produces organic acids, solubilizes orthophosphates and mineralizes phosphorus. These characteristics help to improve the bio-inoculant's performance in soil, since Colombian soils are characterized by presenting organic matter with elevated phosphorous content [38, 77, 78].

4.4. Seed germination and Allium cepa growth

Germination percentage in wet chamber is a fast test (5 days) that allows to determine bio-inoculant positive or negative effect on a particular seed. Additionally, seed immersion in suspension with microorganisms is a frequent practice. The solute can be made of water or water with osmoregulators (osmoconditioning) to accelerate the germination process, determine seed viability and promote a rapid and synchronized establishment of seedlings [65, 79]. According to results illustrated in Figure 3A, co-culture favoured seed germination (91%) at a higher percentage in comparison with individual bacterial strains or controls. This effect could be due to nutrient contribution, substances produced by both bacteria, which promote plant growth and to co-culture solubilized phosphorus. There is evidence supporting fertilization with PSB improves plant growth, since various microorganisms in the soil, including bacteria, improve P supply to the plant, as a result of their capacity to solubilize phosphorus [22]. Taking into account P availability is a limiting step in plant nutrition; suggesting PSB provides a fundamental contribution, thus improves the growth performance of plants [12, 80, 81]. Sridevi & Ramakrishnandevi, (2010) demonstrated co-inoculation with bacteria of the genus Azospirillum and arbuscular mycorrhizal fungi favored germination of *A. cepa* seeds, the establishment seedbeds and plots, due to combined production or promoting plant substances and to greater availability of nutrients, such as phosphorus [9].

Moreover, effect of compounds such as osmotic conditioners to assist in seed germination was reported by Marín Sánchez *et al.*, (2007). In their work with onion seeds they demonstrated turgor degree and percentage of germination varied as a function of the osmoconditioning agent,

osmotic potential and treatment duration [79]. Zhao *et al.,* (2018) reported certain factors, such as mild membrane deterioration or enzyme inactivation of the seed can be reversible, which suggests certain loss of turgor can be recovered through physical, chemical and biological treatments [40], as the addition of bio-inoculants containing bacteria [22, 40]. In contrast to that reported by Marín Sánchez *et al.* (2007), the present work did not utilize osmotic conditioners. However, the possibility cannot be ruled out that complete co-culture administration allowed seed germination greater than 90% after 5 days of evaluation [41, 82]. This co-culrure included microorganisms and residual compounds from 6 h of production diluted in 0.85% saline solution (w/v) at a concentration of 8.0 Log10 CFU mL^{-1} that could have generated in the seed physiological processes associated with pre-germination, such as free radical uptake, enzymatic activation, and membrane recovery.

For the second experiment with seeds the soil employed was characterized by being clay loam with high contents of aluminum and iron, which limit phosphorus availability (Table 3). Never the less, evaluated bacteria had the capacity to solubilize phosphorus, thus exerting a beneficial effect [83] for seed germination and seedling growth, where co-culture treatment was the most efficient. Although the evaluated soil was not supplemented with phosphorus, bacteria had the capacity to make the element accessible.

Available phosphorus concentration before inoculation was high (250 mg kg^{-1}), as well as potassium (289 mg kg^{-1}), therefore the soil was not supplemented with fertilizer at seedtime, since organic acids produced by evaluated bacteria could increase element availability improving plant growth [10, 84]. Balemi *et al.* (2007) reported a significant increase in nutrient percentage in *A. cepa*, due to *Azotobacter* spp., inoculation, which could have facilitated root development, leading to better nutrient absorption [85].

On the other hand, nutrients required for *A. cepa* growth, such as potassium and boron were at medium and low concentrations, which could have limited vegetable development. Ammonium contents were high and nitrates low. This variation in nitrogen forms could be associated with chemical fertilizer use, which in crops are rotated every six months. These types of fertilizers have a much higher nutrient release rate in comparison with organic fertilizers [86].

Under greenhouse conditions, PSB favoured total dry weight and *A. cepa* leaf length, with values of 69 ± 13 mg for co-culture, 50 ± 4 mg for *Pseudomonas* sp., 47 ± 7 mg for *Serratia* sp. and 38 ± 5.0 mg for control. For leaf length observed values were 17.3 ± 0.8 cm, 13.8 ± 0.2 cm and 11.7 ± 0.5 cm long for co-culture, *Pseudomonas* sp., *Serratia* sp. and control, respectively (Figure 3B). López-Dávila *et al.* (2017) reported use of efficient microorganisms as biofertilizers in *Allium cepa* plants, demonstrating efficiency for studied microorganisms in plant growth and development [87]. Batool & Iqbal, (2019) reported on PSB consortia as an alternative for chemical fertilizers, demonstrating PSB capability to produce phytohormones, siderophores, ammonium, hydrogen cyanide, with subsequent increase in seed germination and root length and apparition of *Triticum aestivum* shoot [88]. Ahmad *et al.* (2014) reported PSB use isolated from soils from different crops could improve plant growth. Observed results in this study demonstrated *Pseudomonas* sp., and *Serratia* sp., in consortium potential to increase *A. cepa* growth and their possible effect on vegetable growth promotion [89].

Phosphate solubilizing bacteria exert an important role in plant nutrition, through the absorption of phosphorus. Their use is an important contribution to the bio-fertilization of agricultural crops. Therefore, research in *A. cepa* cultures will continue to assay stability and effect of this bio-inoculant in the field.

In conclusion, *Pseudomonas* sp., and *Serratia* sp., had the capacity to grow in PR, brewer's yeast hydrolysate and low glucose concentration, promoting germination of *A. cepa* seeds. Additionally, co-culture application at 8.0 Log_{10} CFU mL^{-1} twice a week for two months favored *A. cepa* total dry weight increase in comparison with controls.

Declarations

Author contribution statement

Andrea Blanco-Vargas: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Lina M. Rodríguez-Gacha, Natalia Sánchez-Castro: Performed the experiments; Analyzed and interpreted the data.

Rafael Garzón-Jaramillo, Lucas D. Pedroza-Camacho: Performed the experiments.

Raúl A. Poutou-Piñales, Claudia M. Rivera-Hoyos, Lucía A. Díaz-Ariza, Aura M. Pedroza-Rodríguez: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported by Gobernación de Boyacá, Departamento Administrativo de Ciencia y Tecnología e Innovación y COLCIENCIAS Grant No. 733 de 2015 "Training of high level human capital for the Departamento de Boyacá" and the Pontificia Universidad Javeriana Bogotá, D.C. Colombia" grant No. 00008626: Development of a biofertilizer based on biochar and phosphate solubilizing bacteria for Allium cepa culture. Phosphate solubilizing bacteria employed in this study were isolated following resolution No. 00778 of 2017 "Framework permit for the collection of specimens of wild species of biological diversity for non-commercial scientific research purposes granted to the Pontificia Universidad Javeriana by the Ministry of Environment and Sustainable Development". The mobility permit number was 2018033275-1-000.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

Authors thank Mr. Gabriel Argüello for contacting farmers and his disposition to work in this Project. They also manifest their gratitude to Cesar López and Paulino Pérez for allowing sampling in "*Departamento de Boyacá*" soils, Mr. Ferney Viancha for donating *Allium cepa* seeds and María Lucía Gutiérrez, Ph.D., for English revision.

Refererences

[1] F. Silva, C. Nascimento, A. Alvarez, P. Araujo, Inputs of rare earth elements in Brazilian agricultural soils via P-containing fertilizers and soil correctives, J. Environ. Manag. 232 (2019) 90–96.

[2] F. Silva, C. Nascimento, P. Araujo, Environmental risk of trace elements in P-containing fertilizers marketed in Brazil, J. Soil Sci. Plant Nutr. 17 (2017) 635–647.

[3] X. Yu, X. Liu, T. Zhu, G. Liu, C. Mao, Co-inoculation with phosphate-solubilizing and nitrogen-fixing bacteria on solubilization of rock phosphate and their effect on growth promotion and nutrient uptake by walnut, Eur. J. Soil Biol. 50 (2012) 112–117.

[4] H. Yadav, R. Fatima, A. Sharma, S. Mathur, Enhancement of applicability of rock phosphate in alkaline soils by organic compost, Appl. Soil Ecol. 113 (2017) 80–85.

[5] Agencia Nacional de Minería (Colombia), 2015. https://www.anm.gov.co/sites/default/files/DocumentosAnm/roca_fosforica.pdf.

[6] G. De Oliveira Mendes, A. Galvez, M. Vassileva, N. Vassilev, Fermentation liquid containing microbially solubilized P significantly improved plant growth and P uptake in both soil and soilless experiments, Appl. Soil Ecol. 117–118 (2017) 208–211.

[7] I. Mardad, A. Serrano, A. Soukri, Effect of carbon, nitrogen sources and abiotic stress on phosphate solubilization by bacterial strains isolated from a moroccan rock phosphate deposit, J. Adv. Chem. Eng. 4 (2014) 1000102.

[8] M. Manzoor, M.K. Abbasi, T. Sultan, Isolation of phosphate solubilizing bacteria from maize rhizosphere and their potential for rock phosphate solubilization-mineralization and plant growth promotion, Geomicrobiol. J. 34 (2017) 81–95.

[9] S. Sridevi, K. Ramakrishnan, Effects of combined inoculation of am fungi and Azospirillum on the growth and yield of onion (*Allium cepa* L.), J. Phytol. 2 (2010) 88–90.

[10] F. Yoldas, S. Ceylan, N. Mordogan, B.C. Esetlili, Effect of organic and inorganic fertilizers on yield and mineral content of onion (*Allium cepa* L.), Afr. J. Biotechnol. 10 (2011) 11488–11492.

[11] D. Thakur, R. Kaushal, V. Shyam, Phosphate solubilising microorganisms: role in phosphorus nutrition of crop plants-A review, Agric. Rev. 35 (2014) 159–171.

[12] L. Valetti, L. Iriarte, A. Fabra, Growth promotion of rapeseed (*Brassica napus*) associated with the inoculation of phosphate solubilizing bacteria, Appl. Soil Ecol. 132 (2018) 1–10.

[13] S.I. Shedeed, S.A.A. EL-Sayed, D.M. Abo Bash, Effectiveness of bio-fertilizers with organic matter on the growth, yield and nutrient content of onion (*Allium cepa* L.) plants, Eur. Int. J. Sci. Technol. 3 (2014) 115–122.

[14] M. Parhamfar, A. Badoei, M. Parhamfar, S. Fahimi, Purification and characterization of an extracellular phosphatase enzyme from *Bacillus* spp, J. Cell Mol. Res. 8 (2016) 90–97.

[15] R. Jog, M. Pandya, G. Nareshkumar, S. Rajkumar, Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth, Microbiology 160 (2014) 778–788.

[16] M. Stella, M.S. Halimi, Gluconic acid production by bacteria to liberate phosphorus from insoluble phosphate complexes, J. Trop. Agric. Food Sci. 43 (2015) 41–53.

[17] B.C. Behera, et al., Phosphate solubilization and acid phosphatase activity of *Serratia* sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India, J. Genet. Eng. Biotechnol. 15 (2017) 169–178.

[18] J. Zhu, M. Li, M. Whelan, Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: a review, Sci. Total Environ. 612 (2018) 522–537.

[19] A. Zaidi, M.S. Khan, M. Ahemad, M. Oves, Plant growth promotion by phosphate solubilizing bacteria, Acta Microbiol. Immunol. Hung. 56 (2009) 263–284.

[20] M.S. Khan, A. Zaidi, M. Ahemad, M. Oves, P.A. Wani, Plant growth promotion by phosphate solubilizing fungi – current perspective, Arch. Agron Soil Sci. 56 (2010) 73–98.

[21] M. Oves, M.S. Khan, H.A. Qari, *Ensifer adhaerens* for heavy metal bioaccumulation, biosorption, and phosphate solubilization under metal stress condition, J.Taiwan Inst. Chem. Eng. 80 (2017) 540–552.

[22] M. Oves, M.S. Khan, H.A. Qari, Chromium-reducing and phosphate-solubilizing *Achromobacter xylosoxidans* bacteria from the heavy metal-contaminated soil of the Brass city, Moradabad, Int. J. Environ. Sci. Technol. 16 (2019) 6967–6984.

[23] J.P. Angulo-Cortés, A. García-Díaz, A.M. Pedroza, M.M. Martínez-Salgado, V. Gutiérrez-Romero, Diseño de un medio para la producción de un co-cultivo de bacterias fosfato solubilizadoras con actividad fosfatasa, Univ. Sci. 17 (2012) 43–52.

[24] Y.P. Chen, et al., Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities, Appl. Soil Ecol. 34 (2006) 33–41.

[25] B. Hameeda, Y.H.K. Reddy, O.P. Rupela, G.N. Kumar, G. Reddy, Effect of carbon substrates on rock phosphate solubilization by sacteria from composts and macrofauna, Curr. Microbiol. 53 (2006) 298–302.

[26] S. Swetha, T. Padmavathi, Study of acid phosphatase in solubilization of inorganic phosphates by *Piriformospora indic*, Pol. J. Microbiol. 65 (2016) 407–412.

[27] Y. Wei, et al., Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation, Bioresour. Technol. 247 (2018) 190–199.

[28] Juan Wu, et al., Effect of different components of single superphosphate on organic matter degradation and maturity during pig manure composting, Sci. Total Environ. 646 (2019) 587–594.

[29] J.M. Igual, A. Valverde, E. Cervantes, E. Velazquez, Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study, Agronomie 21 (2001) 561–568.

[30] V.M. Karličić, et al., Isolation and characterization of bacteria and yeasts from contaminated soil, J. Agric. Sci. 61 (2016) 247–256.

[31] R.A. Poutou, E. Amador, M. Candelario, Banco de células primario (BCP): Caracterización y papel en la producción de proteínas recombinantes, Biotecnol. Apl. 11 (1994) 55–59.

[32] T. Tatusova, et al., NCBI prokaryotic genome annotation pipeline, Nucl. Acids Res. 44 (2016) 6614–6624.

[33] M.A. Larkin, et al., Clustal W and Clustal X version 2.0, Bioinformation 23 (2007) 2947–2948.

[34] T. Jukes, C. Cantor, Evolution of protein molecules, in: H.N. Munro (Ed.), Mammal. Prot. Metabol., Academic Press, New York, USA, 1969, pp. 21–132.

[35] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, X. Mega, Molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (2018) 1547–1549.

[36] J. Murphy, J.P. Riley, A modified singles solution method for the determination of phosphate in natural waters, Anal. Chim. Acta 27 (1962) 31–36.

[37] G. Miller, Use of dinitrosalicilic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.

[38] C.A. Cisneros Rojas, M. Sánchez de Pragar, J.C. Menjivar Flores, Identificación de bacterias solubilizadoras de fosfatos en un andisol de la región cafetera colombiana, Rev. Colomb. Biotecnol. XIX (2017) 21–28.

[39] H.Ç. Kaymak, İ. Güvenç, F. Yarali, M.F. Dönmez, The effects of bio-priming with PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions, Turk. J. Agric. For. 33 (2009) 173–179.

[40] D. Liu, et al., Effect of fertilizer prepared from human feces and straw on germination, growth and development of wheat, Acta Astronaut. 145 (2018) 76–82.

[41] H. Arjona Díaz, A. Guerrero Sánchez, C.O. Prieto Medina, Estudios de osmoiniciación de semillas de cebolla de bulbo (*Allium cepe* L.), Agron. Col. XV (1998) 143–152.

[42] A. Blanco-Vargas, et al., A novel textile wastewater treatment using ligninolytic co-culture and photocatalysis with TiO2, Univ. Sci. 23 (2018) 437–464.

[43] M. Rashid, S. Khalil, N. Ayub, S. Alam, F. Latif, Organic acids production and phosphate solibilization by phosphate solubilizing microorganism (PSM) under *in vitro* conditions, Pak. J. Biol. Sci. 7 (2004) 187–196.

[44] G.J. Bouyoucos, Hydrometer method improved for making particle size analyses of soils, Agron. J. 54 (1962) 464–465.

[45] A. Walkley, I.A. Black, An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method, Soil Sci. 37 (1934) 29–38.

[46] J. Kjeldahl, A new method for the determination of nitrogen in organic bodies, Z. Anal. Chem. 22 (1883) 366–382.

[47] A. Mehlich, Determination of P, Ca, Mg, K, Na, and NH4", North Carolina Soil Test Division. Mimeo, Raleigh, North Carolina, 1953.

[48] R. Bray, L. Kurtz, Determination of total, organic, and available forms of phosphorus in soils, Soil Sci. 59 (1945) 39–46.

[49] M.A. Tabataba, J.M. Bremner, Use of p-nitrophenyl phosphate for assay of soil phosphatase activity, Soil Biol. Biochem. 1 (1969) 301–307.

[50] C. Álvarez-López, W. Osorio-Vega, M.C. Díez-Gómez, M. Marín-Montoya, Caracterizaciónn bioquímica de microorganismos risosféricos de plantas de vainilla con potencial como biofertilizantes Agron, Mesoam 25 (2014) 225–241.

[51] A. Fox, W. Kwapinski, B.S. Griffiths, A. Schmalenberger, The role of sulfur- and phosphorus-mobilizing bacteria in biochar-induced growth promotion of *Lolium perenne*, FEMS Microbiol. Ecol. 90 (2014) 78–91.

[52] A. Babu, P. Shea, D. Sudhakar, I. Jung, B. Oh, Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal(loid)-contaminated mining site soil, J. Environ. Manag. 151 (2015) 160–166.

[53] G. Kaur, M. Reddy, Effects of phosphate-solubilizing bacteria, rock phosphate and chemical fertilizers on maize-wheat cropping cycle and economics, Pedosphere 25 (2015) 428–437.

[54] Y.S. Gusain, R. Kamal, C.M. Mehta, U.S. Singh, A.K. Sharma, Phosphate solubilizing and indole-3-acetic acid producing bacteria from the soil of Garhwal Himlaya aimed to improve the growth of rice, J. Environ. Biol. 36 (2015) 301–307.

[55] N. Oteino, et al., Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates, Front. Microbiol. 6 (2015) 745.

[56] R. Paul, et al., Phosphorus dynamics and solubilizing microorganisms in acid soils under different land uses of Lesser Himalayas of India, Agrofore. Sys. 92 (2018) 449–461.

[57] S.A. Omar, The role of rock-phosphate-solubilizing fungi and vesicular±arbusular-mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate, World J. Microbiol. Biotechnol. 14 (1997) 211–218.

[58] P. Gupta, V. Kumar, Value added phytoremediation of metal stressed soils using phosphate solubilizing microbial consortium, World J. Microbiol. Biotechnol. 33 (2017) 9.

[59] G. De Oliveira Mendes, et al., Biochar enhances *Aspergillus niger* rock phosphate solubilization by increasing organic acid production and alleviating fluoride toxicity, Appl. Environ. Microbiol. 80 (2014) 3081–3085.

[60] Z. Liu, et al., Characterization of phosphate-solubilizing bacteria isolated from calcareous soils, Appl. Soil Ecol. 96 (2015) 217–224.

[61] S. Alam, S. Khalil, N. Ayub, M. Rashid, In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere, Int. J. Agric. Biol. 4 (2002) 454–458.

[62] N. Kamou, et al., Investigating the compatibility of the biocontrol agent *Clonostachys rosea* Ik726 with prodigiosin-producing *Serratia rubidaea* S55 and phenazine-producing *Pseudomonas chlororaphis* ToZa7, Arch. Microbiol. 198 (2016) 369–377.

[63] D. Paul, S.N. Sinha, Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa K*UPSB12 with antibacterial potential from river Ganga, India, Ann. Agrar. Sci. 15 (2017) 130–136.

[64] M. Ludueña, et al., Effects of P limitation and molecules from peanut root exudates on *pqq*E gene expression and pqq promoter activity in the phosphate-solubilizing strain *Serratia* sp. S119, Res. Microbiol. 168 (2017) 710–721.

[65] L. Pulido, A. Cabrera, N. Medina, Biofertilization using rhizobacteria and amf in the production of tomato (*Lycopersicon esculentum* Mill.) and onion (*Allium cepa* L.) seedlings. II. root colonization and nutritional status, Cultiv. Trop. 24 (2003) 5–13.

[66] B. Hameeda, Y.H. Reddy, O.P. Rupela, G.N. Kumar, G. Reddy, Effect of carbon substrates on rock phosphate solubilization by bacteria from composts and macrofauna, Curr. Microbiol. 53 (2006) 298–302.

[67] Y. Chen, et al., Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities, Appl. Soil Ecol. 34 (2006) 33–41.

[68] R. An, L.A. Moe, Regulation of pyrroloquinoline quinone-dependent glucose dehydrogenase activity in the model rhizosphere-dwelling bacterium *Pseudomonas putida* KT2440, Appl. Environ. Microbiol. 82 (2016) 4955–4964.

[69] M. Della, M. Godoy, A. Godeas, J. Scervino, Fungal extracellular phosphatases: their role in P cycling under different pH and P sources availability, J. Appl. Microbiol. 124 (2017) 155–165.

[70] E.J. Serna Posso, M. Sánchez de Prager, C.A. Cisneros Rojas, Organic acids production by rhizosphere microorganisms isolated from a typic melanudands and its effects on the inorganic phosphates solubilization, Acta Agr 66 (2017) 241–247.
[71] B. Sreenivasulu, K. Chalapathi, C. Paramageetham, G. Prasada Babu, In Vitro phosphate solubilization abilities of three indigenous bacteria isolated from *Muscovite mine*, Int. J. Curr. Microbiol. App. Sci. 3 (2014) 135–143.
[72] A.E. Richardson, R.J. Simpson, Soil microorganisms mediating phosphorus availability update on microbial phosphorus, Plant Physiol. 156 (2011) 989–996.
[73] B. Behera, S. Singdevsachan, R. Mishra, S. Dutta, H. Thatoi, Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove—a review, Biocatal. Agricul. Biotechnol. 3 (2014) 97–110.
[74] P.U. Krishnaraj, S. Dahale, Mineral phosphate solubilization: concepts and prospects in sustainable agriculture, Proc. indian Natl. Sci. Acad. 80 (2014) 389–405.
[75] C. Lara Mantilla, J.L. Negrete Peñata, Efecto de un bioinoculante a partir de consorcios microbianos nativos fosfato solubilizadores, en el desarrollo de pastos Angleton (*Dichantium aristatum*), Rev. Colomb. Biotecnol. XVII (2015) 122–130.
[76] A.P. Nandimath, D.D. Karad, S.G. Gupta, A.S. Kharat, Consortium inoculum of five thermo-tolerant phosphate solubilizing Actinomycetes for multipurpose biofertilizer preparation, Iran. J. Microbiol. 9 (2017) 295–304.
[77] I.G.A.C., IGAC, *Estudio General De Suelos Y Zonificación De Tierras Del Departamento De Boyacá*, 2005.
[78] O.T.D.d.B., OTDB, Productividad sector agropecuario. Documento de diagnóstico en construcción, Gobernación de Boyacá. Tunja, 2018, 2018.
[79] J. Marín Sánchez, J.A. Mejía Contreras, A. Hernández Livera, A. Carballo Carballo, A. Peña Lomelí, Acondicionamiento osmótico de semillas de cebolla (*Allium cepa* L.), Agric. Tec. Mex. 33 (2007) 63–71.
[80] H. Rodríguez, R. Fraga, Phosphate solubilizing bacteria and their role in plant growth promotion, Biotechnol. Adv. 17 (1999) 319–339.
[81] M.M. Yazdani, M.A. Bahmanyar, H. Pirdashti, M.A. Esmaili, Effect of phosphate solubilisation microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of Corn (*Zea mays* L.), Int. J. Agricult. Biosys. Eng. 3 (2009) 50–52.
[82] Y. Zhao, M. Hu, Z. Gao, X. Chen, D. Huang, Biological mechanism of a novel hydro-electro hybrid priming recovers potential vigor of onion seeds, Environ. Exp. Bot. 150 (2018) 260–271.
[83] P.K.S. Jayathilake, I.P. Reddy, D. Srihari, K.R. Reddy, Productivity and soil fertility status as influenced by integrated use of N-fixing biofertilizers, organic matures and inorganic fertilizers in onion the, J. Agric. Sci. 2 (2006) 46–58.
[84] G.M. Khan, S. Gupta, S. Banarg, Studies on the solubilization of phosphorus in presence of different city wastes, J. Indian Soc. Soil Sci. 29 (1981) 120–122.
[85] T. Balemi, N. Pal, A.K. Saxena, Response of onion (*Allium cepa* L.) to combined application of biological and chemical nitrogenous fertilizers, Acta Agric. Slov. 89 (2007) 107–114.
[86] T.H. Seran, S. Srikrishnah, M.Z. Ahamed, Effect of different levels of inorganic fertilizers and compost as basal application on the growth and yield of onion (*Allium cepa* L.), J. Agric. Sci. 5 (2010) 64–70.
[87] E. López-Dávila, Z. Gil-Unday, D. Henderson, A. Calero-Hurtado, J. Jiménez-Hernández, Uso de efluente de planta de biogás y microorganismos eficientes como biofertilizantes en plantas de cebolla (*Allium cepa* L., cv "Caribe-71"), Cult. Trop 38 (2017) 7–14.
[88] S. Batool, A. Iqbal, Phosphate solubilizing Rhizobacteria as alternative of chemical fertilizer for growth and yield of *Triticum aestivum* (Var. Galaxy 2013), Saudi J. Biol. Sci. 26 (2019) 1400–1410.
[89] E. Ahmad, A. Zaidi, M.S. Khan, Response of PSM inoculation to certain Legumes and cereal crops, in: M. Khan, A. Zaidi, J. Musarrat (Eds.), Phosphate Solubilizing Microorganisms, Springer, Cham, 2014, pp. 175–205.