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

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**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 68.1 ± 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 × 10⁸ 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 Log₁₀ 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 (P₂O₅) content varies between 20 and 30 % (w/v), [5]; however, despite its high phosphorous content it is...
found as an unexchangeable form with low solubility at neutral and alkaline pH [6]. Therefore, it is not an available phosphorus source for plants, requiring chemical or microbiological pretreatment to favor insoluble nutrient transfer, promoting phosphorus solubilization and plant absorption [7, 8].

Combined use of PR and phosphorus 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 fruit, since P is required for seed germination, early root growth and bulb thickening [9, 10].

Phosphorus 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., *Buchnerella* spp., *Achromobacter* spp., *Acrobacter* spp., *Serratia* spp., *Erwinia* spp., and *Pseudomonas* 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 bio-inoculate. PSB culture can use different organic and inorganic phosphorus sources, such as PR, tri-calcium phosphate, agriindustry 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* spp., 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 5°47’03.5”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/](https://www.ncbi.nlm.nih.gov/)) [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/](https://www.ncbi.nlm.nih.gov/)) and RDP (Ribosomal Database Project, [http://rdp.cme.msu.edu](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 (Calibey®; [http://www.calibey.co](http://www.calibey.co)), (25 % P₂O₅, 32 % CaO, 14 % SiO₂, 0.5 % Al₂O₃ (w/w), 10 g L⁻¹ glucose, 0.5 g L⁻¹ yeast extract, 0.5 g L⁻¹ (NH₄)₂SO₄, 0.2 g L⁻¹ KCl, 0.3 g L⁻¹ MgSO₄, 0.004 g L⁻¹ MnSO₄, 0.0004 g L⁻¹ FeSO₄, 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].

\[
SI = \frac{A}{B}
\]

(1)

where: A is the colony halo in mm + solubilization halo diameter in mm, B colony diameter (mm). Results correspond to average of three replicates ± 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 flasks 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 × 10^6 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 × 10^6 cells ml⁻¹ were placed on top of the agar for interaction determination. For negative control filter paper disk impregnated with dH₂O was used. Petri dishes were incubated at 30 °C for 48 h. Antagonism was determined by mass absence or 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 (NH₄)₂SO₄, 0.2 g L⁻¹ KCl, 0.3 g L⁻¹ MgSO₄, 0.004 g L⁻¹ MnSO₄, 0.0004 g L⁻¹ FeSO₄ 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 (NH₄)₂SO₄, 0.2 g L⁻¹ KCl, 0.3 g L⁻¹ MgSO₄, 0.004 g L⁻¹ MnSO₄, 0.0004 g L⁻¹ FeSO₄ 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).
where phosphates concentration by using a standard curve (0.5 M NaOH and 86

2.5. Determination of phosphatase activity

phosphate or phosphorus in solution was observed, respectively.

product, \( T \) is time where the greatest amount of product expressed as

(0.01 μmol mL\(^{-1}\)).

Moreover, phosphorus yield in glucose (Equation 2), biomass (measured as CFU mL\(^{-1}\)) volumetric productivity (Equation 3), phos-

phosphate activity and β-phosphate (Equation 4) productivity were calcu-

ded. To determine possible productivity and yield significant differences, a comparison of means (mean ± SD) between culture media was made (α = 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.

\[
Y(p/s) = \frac{P_f - P_i}{S_0 - S_f}
\]

where: \( Y(p/s) \) is the product yield (phosphorus in solution) divided by the substrate (glucose), \( P_i \) is a final product (soluble phosphorus released from the solubilization of the PR) concentration, \( P_i \) initial product concentration, \( S_0 \) initial substrate concentration, \( S_f \) final substrate concentration.

\[
P(x) = \frac{X_f - X_0}{T}
\]

where: \( P(x) \) is the biomass productivity, \( X_i \) is final biomass concentration, \( X_0 \) is initial biomass concentration, \( T \) is time where the maximum biomass concentration was obtained

\[
P(p) = \frac{P_f - P_0}{T}
\]

where: \( P(p) \) is the product productivity \( P_i \) 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-nitrophenol phosphatase 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-nitro-

phenol 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\(_2\)SO\(_4\) 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\(_2\)O 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; at time 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\(_2\)O. 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 infloresc-

ence 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 signif-

icant 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 Pseudomo-

nas with 99 % homology in 100 % of its entire sequence in compar-

ison with Pseudomonas koreensis and Pseudomonas moravensis. 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/16S r-

ibosomal 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 quinquorans and Serratia grimesii. These results were in
agreement with phylogenetic analysis, where this strain was not partic-
ularly grouped with any species (Figure 1B). Considering C7 strain
demonstrated a high phosphate solubilization index, it could be identi-
fied as *Serratia liquefaciens*, 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
coculture production. For preliminary growth and solubilization assays
in SMRS1 supplemented with 5 g L\(^{-1}\) PR, soluble phosphorus concentra-
tions for each individual bacteria strain were 66.2 and 4.7 mg L\(^{-1}\)
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 illus-
trated 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 present, for *Pseudomonas* sp., growth,
some percentages of organic acids production 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 (\(p = 0.96,
\)

![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.](image-url)
p < 0.0012). In contrast, SP was negatively correlated with pH (ρ = -0.90, p < 0.0010) and residual glucose (ρ = -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 1. Preliminary solubilization results and Gauze interactions.

| Strain         | Solubilization Index | Inhibition halos (mm) | Soluble phosphorus (mg L⁻¹) | Log₁₀ 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.
Phosphatase activity was only quantified in MT11B media, where a gradual increase was observed until reaching a maximum volumetric activity of 1.3 ± 0.04 UP at 6 h of culture, with a gradual decrease to end with an activity of 0.21 ± 0.01 UP at 12 h (Figure 2A). Each bacterial strain presented alkaline phosphatase activity of 2.9 ± 0.01 and 0.01 ± 0.03 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 (*p = 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 dH₂O and 83 % for MT11B at 1/1000 dilution. These results showed employing co-culture at 8.0 Log10 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 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 x 10^5 ± 7 x 10^4 | 4 x 10^5 ± 3 x 10^4 |
| 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 ± 0.03b | 32.9 ± 1.3a |
| Y (Soluble phosphorus/CFU) mg mg⁻¹ | 0.065 ± 0.03*a,b | 0.04 ± 0.002b |
| Y (Soluble phosphorus/glucose) mg mg⁻¹ | 2 x 10⁻¹² ± 5 x 10⁻¹⁴b | 4 x 10⁻¹² ± 2 x 10⁻¹⁸a |

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

### 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 cm⁻³   | Calculated         |
| Organic carbon     | 3.99  | (%)      | Colorimetric [45]  |
| Organic matter     | 6.87  | (%)      | Calculated         |
| Total nitrogen     | 0.32  | (%)      | Micro-Kjeldahl [46] |
| C/N ratio          | 12    |          | Calculated         |
| Exchangeable potassium | 289  | mg kg⁻¹  | Ac. NH₄/atomic absorption |
| Exchangeable sodium | 60   | mg kg⁻¹  | Ac. NH₄/atomic absorption |
| Iron               | 42    | mg kg⁻¹  | Acid mix/atomic absorption/Melhich 1 [47] |
| Biron              | 0.49  | mg kg⁻¹  | Ca(OH)PO₄/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 |
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$_2$O 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 ($69 \pm 13$ mg ($p = 0.0001$). In the individual application of the isolates, the total dry weight for Pseudomonas sp., was 50 $\pm$ 4 mg ($p = 0.0023$) and for Serratia sp., it was 47 $\pm$ 7 mg ($p = 0.0030$). Last, for control A. cepa total dry weight 38 $\pm$ 5.0 mg ($p = 0.0078$). The leaf height inoculated with co-culture was 17.3 $\pm$ 0.8 cm, followed by plants watered with Pseudomonas sp. (13.8 $\pm$ 0.2 cm) and Serratia sp. (11.7 $\pm$ 0.5 cm). Last, seedlings watered with dH$_2$O for two months had an average leaf height of 15.4 $\pm$ 1.9 cm (Figure 3B).

Total heterotrophic bacteria count in microbiological analyses in soil samples showed counts were similar and ranged between 5.0 $\pm$ 1.0 and 5.4 $\pm$ 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 $\pm$ 0.7 and 4.6 $\pm$ 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 $\pm$ 0.2), reaching values of 5.6, 5.2, 5.6 and 5.4 $\pm$ 0.2 for co-culture, Pseudomonas sp., Serratia sp. and control with dH$_2$O 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
chemical structure, molecular weight, or diffusion velocity \cite{7, 19}; even
be due to variations between bacteria on the type of acid produced,
must not only be based on SI, considering other criteria must be taken
outside of the cell. Various authors have reported similar results in trying
though all acids produced by bacteria are water soluble and are excreted
acids are produced during carbohydrate metabolism, such as glucose,
organic acid production, such as gluconic, oxalic, citric and malic acid
Pseudomonas sp. and Serratia sp., \cite{17}. Alam \textit{et al.} (2017) obtained 9.0 logarithmic units after 20 h in NBRIP media with tricalcium phosphate \cite{64}, one logarithmic unit
less than for MT11B media with 12 h more hours of culture. Chen \textit{et al.}
(2006) cultured for 3 days Gram-negative bacteria in mineral media supplemented with 5.0 g L$^{-1}$ tricalcium phosphate and obtained \textit{Serratia marcescens} counts between 8.7 and 9.3 logarithmic units \cite{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 \textit{Pseudomonas} and \textit{Serratia} can employ
different sources of carbon and nitrogen (organic and inorganic) and
phosphorus (tricalcium phosphate, hydroxyapatite, iron phosphate,
aluminum phosphate and PR \cite{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 \cite{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 \cite{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-
in co-inoculation and possibly during bio-inoculant production and
further use at greenhouse level, maintaining positive or neutral interac-
tion \cite{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$^{-1}$ \cite{24, 52, 64}. When obtaining elevated
biomass concentrations, the bio-inoculant could be dosed, in such way as
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 \cite{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 \cite{4, 7, 66}. Among the factors
considered are media composition associated with sources of carbon,
nitrogen, phosphorus, raw material costs and time of processes. There-
fore, it was necessary to evaluate alternative media different from SMR1,
Pikovskaya and NBRIP (\textit{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 \textit{Pseudo-
onas} 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 \textit{Serratia} sp. These results could be associated with \textit{Pseudo-
onas} sp. reduced capacity to solubilize phosphorus. In preliminary as-
says \textit{Pseudomonas} sp. produced 66.2 mg L$^{-1}$ phosphorus in comparison with \textit{Serratia} sp. with 89.5 mg L$^{-1}$ (Table 1).

Colony counts were similar to those reported by other authors \cite{23},
who used PR and \textit{Sacharomyces cerevisiae} hydrolysate with a 24 h pro-
duction 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 \textit{et al.} (2017) obtained 9.0 logarithmic units after 20 h
in NBRIP media with tricalcium phosphate \cite{64}, one logarithmic unit
less than for MT11B media with 12 h more hours of culture. Chen \textit{et al.}
(2006) cultured for 3 days Gram-negative bacteria in mineral media supplemented with 5.0 g L$^{-1}$ tricalcium phosphate and obtained \textit{Serratia marcescens} counts between 8.7 and 9.3 logarithmic units \cite{67}.
culture consumes more efficiently glucose when initial concentration is $> 10 \text{ g L}^{-1}$. Under this condition glucose consumption velocity was 0.612 h$^{-1}$, a higher value in comparison with SMRS1 media (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 phosphate 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 (P) 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 orthophosphates 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 only evident 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 favorable 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-Martíll et al., (2015) and Pandimath et al., (2017) demonstrated bacteria consortia use at $10 \times 10^6$ CFU mL$^{-1}$ compared with individual strains and in co-culture increases phosphorus solubilization [75, 76].

Organic phosphorus mineralization 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 agreement 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-inoculant 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 & Ramakrishnanadevi, (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 vigor 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-culture 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 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). Lopez-Davila 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-Ariz, Aura M. Pedroza-Rodríguez: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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