

Bioinoculant production composed by *Pseudomonas* sp., *Serratia* sp., and *Kosakonia* sp., preliminary effect on *Allium cepa* L., growth at plot scale

Andrea Blanco-Vargas^{1,2,3}, Lina M. Rodríguez-Gacha¹, Natalia Sánchez-Castro¹, Laura Herrera-Carlosama¹, Raúl A. Poutou-Piñales², Lucía A. Díaz-Ariza³, Viviana Gutiérrez-Romero⁴, Claudia M. Rivera-Hoyos^{1,2}, Leidy D. Ardila-Leal^{1,2}, Aura M. Pedroza-Rodríguez*¹

Selection of phosphate solubilizing bacteria for consortium design

A total of 52 phosphate solubilizing bacteria (PSB) colonies were selected in SMRS1 media from 37 soil samples. These PSB revealed a solubilization halo around them and growth medium acidification, as evidenced from the color change from purple to yellow. The 52 PSB colonies were isolated by sub-culture in SMRS1 agar modified with phosphate rock (PR), (Calboy[®]; www.calboy.co, 2018), (25 % P₂O₅, 32 % CaO, 14 % SiO₂, 0.5 % Al₂O₃ w/v) as the source of phosphorus at a 5 g L⁻¹ concentration (SMRS1-PR). Petri dishes were incubated for 72 h at 30 °C. To select Gram negative bacteria, Gram stain was performed, where 51 (98 %) corresponded to Gram negative bacilli. Solubilization index (SI) was performed in SMRS1-PR agar through the microdrop technique following Blanco-Vargas *et al.* (2020). Bacteria that presented SI > 2.0 were selected for antagonism assays, performed in nutrient agar. Statistical analysis revealed 28 of them (54 %) exhibited antagonism (Blanco-Vargas *et al.* (2020)). Therefore, the remaining 18 (35 %) that did not show antagonism among them were employed for preliminary solubilization assays in SMRS1-PR liquid media for 72 h. Biomass production (CFU mL⁻¹) ($p < 0.000$), soluble P release (mg L⁻¹) ($p < 0.0002$), residual glucose (g L⁻¹) ($p < 0.000$), and media pH ($p < 0.000$) were evaluated as response variables. From these results six bacterial strains were selected (12 %) presenting counts > 1.0 × 10⁷ CFU mL⁻¹ that released > 48 mg L⁻¹ soluble P (SP), (**Table 1**). These PSB were identified by MALDI-TOF, classifying them as follows: three belonged to the *Enterobacter* genus, thus they were not included in this study. Three bacteria were selected (6 %) corresponding to the genera *Pseudomonas* sp., *Serratia* sp., and *Kosakonia* sp. Molecular identification of these PSB has been already reported (Blanco-Vargas *et al.* (2020)). Three selected PSB strains were seeded in DNase- and Blood-agar to rule out pathogenicity. All statistical analyses were performed with STATISTIX version 9.0[®] software with a 95 % confidence interval.

Table 1: Phosphate solubilizing bacteria count and soluble P release at 72 h of culture in SMRS1-PR liquid media

BACTERIA	PSB COUNT (CFU mL ⁻¹)	SOLUBLE P (mg L ⁻¹)
<i>Pseudomonas</i> sp.	(1.2 ± 1.1) × 10 ⁹	66.200 ± 13.357
<i>Serratia</i> sp.	(4.3 ± 5.3) × 10 ⁸	89.500 ± 4.692
<i>Kosakonia</i> sp.	(1.00 ± 0.95) × 10 ¹¹	48.200 ± 9.479
<i>Enterobacter</i> sp.	(4.5 ± 5.3) × 10 ⁹	87.0 ± 18.0
<i>Enterobacter</i> sp.	(2.4 ± 3.0) × 10 ¹¹	82.1 ± 10.1
<i>Enterobacter</i> sp.	(0.95 ± 1.20) × 10 ⁹	55.4 ± 0.0

References

- Blanco-Vargas A, Rodríguez-Gacha LM, Sánchez-Castro N, Garzón-Jaramillo R, Pedroza-Camacho LD, Poutou-Piñales RA, Rivera-Hoyos CM, Díaz-Ariza LA, Pedroza-Rodríguez AM. Phosphate-solubilizing *Pseudomonas* sp., and *Serratia* sp., co-culture for *Allium cepa* L. growth promotion, *Heliyon*, 6 (10): e05218, 2020.
<http://doi.org/10.1016/j.heliyon.2020.e05218>