

In vitro assessment of inorganic phosphate-solubilizing potential of *Bacillus*, *Paenibacillus*, *Peribacillus*, and *Alkalihalobacillus* strains

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Bacillus is a genus of bacteria that has stood out as a plant biostimulant. The sporulation capacity and easy cultivation of *Bacillus* spp. are attractive for their practical use as inoculants and provide a solution to the problem of survival in formulations and under field conditions. We evaluated *in vitro* different *Bacillus*, *Paenibacillus*, *Peribacillus*, and *Alkalihalobacillus* strains for their ability to solubilize insoluble inorganic phosphate sources [Ca₃(PO₄)₂; AlPO₄; FePO₄]. A total of 217 endophytic, epiphytic, and rhizospheric strains isolated from native and cultivated plant species from different Brazilian biomes were obtained from the "Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries (CMMMAABio)". The *B. pumilus* BRM033255 strain was used as positive control. All 217 bacteria were inoculated (quadruplicate) in solid NBRIP-Ca and NBRIP-Al culture media and incubated at 28°C for 15 days. The data on colony diameters and solubilization halos were collected, and the solubilization index (SI) value was calculated as the ratio between the diameter of the clear zone and the colony. For tests in liquid media, 187 of the 217 strains were inoculated (one replicate) in 250 mL conical flasks containing 50 mL of the NBRIP-Ca, NBRIP-Al, and NBRIP-Fe culture media, with an initial optical density adjusted to 0.2 (OD_{600nm}). The flasks were incubated at 30°C and 200 rpm, and aliquots were collected after 3, 6, and 10 days to quantify soluble phosphate in the supernatant using standard colorimetric method and a spectrometric method (inductively coupled plasma optical emission spectrometry, ICP-OES). Based on the results of the primary screening, 21 strains were selected for further characterization. Of the 217 bacteria, 70 showed mean SI values greater than 1 in the Ca₃(PO₄)₂ medium, while none of the strains solubilized AlPO₄. In the screening of 187 bacteria in liquid media, 32 showed soluble phosphate values in the supernatant greater than 100 mg/L in the Ca₃(PO₄)₂ culture medium, with similar values quantified by colorimetric and spectrometric methods. In the assay with 21 selected strains (triplicate), the soluble phosphate in the supernatant reached 449.04 mg/L in the Ca₃(PO₄)₂ medium, 65.59 mg/L in the AlPO₄ medium, and 72.79 mg/L in the FePO₄ medium after 6 days, for *Bacillus* sp. RZ5MS13, *Bacillus* sp. RZ2MS23, and *B. australimaris* BRM052622, respectively. The selected strains will be evaluated for their potential to promote the growth of pre-sprouted sugarcane seedlings.

Key words: *Bacillus*; Inorganic Phosphorus Sources; Sugarcane; Bioprospecting.

Avaliação *in vitro* do potencial de solubilização de fosfato inorgânico de linhagens de *Bacillus*, *Paenibacillus*, *Peribacillus* e *Alkalihalobacillus*

Este estudo avaliou várias cepas de *Bacillus* e outros gêneros quanto à capacidade de solubilizar fosfato inorgânico insolúvel. Foram avaliadas 217 cepas de diferentes biomas brasileiros obtidas da "Coleção de Microrganismos e Microalgas Aplicadas à Agroenergia e Biorrefinarias (CMMMAABio)" e testes *in vitro* revelaram que algumas das mesmas apresentaram capacidade de solubilização em meios sólidos e líquidos. Destacam-se as cepas *Bacillus* sp. RZ5MS13, *Bacillus* sp. RZ2MS23 e *B. australimaris* BRM052622. Essas cepas selecionadas serão avaliadas quanto à sua capacidade de promover o crescimento de mudas de cana-de-açúcar.

Palavras-chave: *Bacillus*; Fontes Inorgânicas de Fósforo; Cana-de-açúcar; Bioprospecção.

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