In vitro assessment of the insoluble phosphate-solubilizing/mineralizing potential of endophytic bacteria from mangrove plants

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Embrapa Agroenergia maintains the Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries (CMMAABio), with more than 10,000 microorganisms from all Brazilian biomes, for example, endophytic bacteria isolated from halophytic plants in the mangrove ecosystem. This ecosystem is characterized by periodic tidal flooding, making environmental factors such as salinity and nutrient availability highly variable, which results in unique ecological conditions. Because of this, mangroves provide a distinct environment and are home to diverse groups of microorganisms that can be explored for biotechnological potential, such as the presence of genes that confer tolerance to high salinity, as well as strains that produce enzymes, proteins, and metabolites involved in the cycling of nutrients such as phosphorus, nitrogen, and others. To add value to conserved microbial biodiversity, the objectives of this work were: (i) to investigate the ability of these bacteria to solubilize/mineralize insoluble inorganic/organic sources of phosphorus; (ii) obtain a DNA bank for research purposes and taxonomic identification of the strains. The present work evaluated 180 bacteria isolated from *Avicennia schaeuriana*, *Laguncularia racemosa*, and *Rhizophora mangle* from a mangrove area impacted by an oil spill in Bertioga/SP. They were reactivated from the stock in Tryptone Soy Agar and incubated at 28°C for up to 7 days. The ability to solubilize insoluble inorganic [$Ca_3(PO_4)_2$; $AlPO_4$; $FePO_4$] and organic (hydrated phytic acid sodium salt) phosphate sources were investigated in solid media. All 180 bacteria were inoculated (triplicate) in the following culture media: NBRIP-Ca, NBRIP-Al, NBRIB-Fe, and phytase screening medium. They were incubated at 28ºC for 15 days. The data on colony diameters and solubilization halos (or enzymatic degradation halos) were collected, and the solubilization index (SI) (or enzymatic index, EI) value was calculated as the ratio between the diameter of the clear zone and the colony. The genomic DNA of the 180 bacteria was extracted for subsequent taxonomic identification using molecular methods. Of the 180 bacteria, 159 showed mean SI values greater than 1 in the $Ca_3(PO_4)_2$ medium, while none of the strains solubilized $AlPO_4$ and $FePO_4$. In the phytase screening medium, 164 strains show mean EI values greater than 1. The bacteria identified (*Enterobacter hormaechei*, *Enterobacter cloacae*, and *Chryseobacterium profundimaris*) represent new strains of Brazilian biodiversity capable of solubilizing/mineralizing insoluble phosphate sources.

Key words: Endophytic Bacteria; Mangrove; Phytase; Phosphate

Avaliação *in vitro* do potencial de solubilização/mineralização de fosfato insolúvel por bactérias endofíticas de plantas de manguezais

Uma coleção de 180 linhagens de bactérias endofíticas de plantas de manguezal foi caracterizada quanto à capacidade de solubilizar/mineralizar fontes insolúveis de fosfato. Das 180 bactérias, 159 apresentaram valores de índice de solubilização superiores a 1 no meio contendo $Ca_3(PO_4)_2$, enquanto nenhuma das bactérias solubilizou $AlPO_4$ e $FePO_4$. No meio contendo fitato, 164 linhagens apresentam valores de índice enzimático de fitase superiores a 1. As bactérias identificadas (*Enterobacter hormaechei*, *Enterobacter cloacae* e *Chryseobacterium profundimaris*) representam novas cepas da biodiversidade brasileira capazes de solubilizar/mineralizar fontes de fosfato insolúveis.

Palavras-chave: Bactérias endofíticas; Manguezal; Fitase; Fosfato

Acknowledgements*: We thank CNPq for funding the Project Number 406335/2022-2. LBM is grateful to Embrapa for the fellowship. CQK and BLM are grateful to CNPq for the fellowships. We are grateful to Thais D. Mendes and Gláucia E. O. Midorikawa for technical support.*