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Quantification of siderophores production by endophytic microorganisms in maize F Batista¹, M Oliveira², T Fernandes¹, V Ribeiro³, I Marriel², E Gomes², U Lana², C Oliveira²

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Tropical soils in agricultural areas of Brazil typically presents acidic condition and are rich in iron phosphate (P-Fe), which results in low availability of phosphorus for plants. Some microorganisms present in the soil or which inhabit the interior of the plants (endophytes) are capable of solubilizing non-available forms of P fixed in iron phosphate through siderophore production, which are organic molecules of low molecular weight with high iron affinity. Therefore, the aim of this study was to quantify the siderophores production by endophytic bacteria isolated from maize in order to identify efficient strains that can be used in future studies of P-Fe solubilization in soil. Quantitation of siderophores was carried out with the chrome azurol S (CAS) reagent in a spectrophotometric assay. Thirteen bacterial isolates belonging to the Embrapa Maize and Sorghum CMMF culture collection of multifunctional and phytopathogenic microorganisms were reactivated on Potato-dextrose Agar (PDA) and incubated at 28°C for 2 days. After growth, bacterial suspensions (OD540nm = 0.5) were prepared in 0.85% saline and 20 µL of each suspension was inoculated in triplicate into 1 ml of nutrient broth. Non-inoculated culture media were added to the 96-well culture plate as negative control. Plates were prepared and incubated for 24 h, 48 h or 72 h at 28 °C under agitation (200 rpm). After each time, a plate was centrifuged for 10 min at 4000 g and 150 µl samples of the supernatants were transferred to a flat bottom 96-well plate with 150 µL of CAS reagent added to each well. The plate was incubated in the dark for 20 minutes at room temperature, and absorbance was read at 630 nm on a spectrophotometer. On each plate a standard curve was added containing 0 to 30 µM EDTA. The results indicated that at least 5 bacteria showed significant siderophore production (Tukey test, p>0,05), in all evaluated time-points. The higher level of siderophore production was obtained after 72h of growth, when especially the bacteria B6 and B10 have reached a siderophore production of 18 uM and 16 uM, respectively. These bacteria represent potential candidates for use in future studies aiming to improve phosphorus acquisition efficiency in maize cultivated in tropical soil with high iron phosphate level.

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