Phosphate solubilizing microorganisms closely associated with maize rhizosphere

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Introduction

The strategy of eliminating the low P fertility production constraint in acid soils with corrective applications of P is limited technically due to the high rates of P fertilizer required and the high P fixing capacity of the soil. Bacterial, actinomyces and fungi can solubilize and mineralize P from inorganic and organic pools of total soil P (Richardson, 2001; Wakelin et al., 2004). The availability of new technologies for studying co-operative microbial interactions and their genetic control in the rhizosphere guarantees a greater management of soil microbial populations, by the development of more effective microbial inoculants, or through the genetic manipulation of specific P-solubilizing microorganisms and/or plant species, which will facilitate their successful applications in agriculture and biotechnology, being considerable benefit, economically and environmentally (Whitelaw, 2000; Richardson , 2001; Barea et al., 2005). The aim of this work was to investigate both the occurrence and levels of phosphate solubilization activity of microorganisms associated with the rhizosphere of maize grown in Brazilian "Cerrado", under conventional tillage and no-tillage managements. The work forms part of our efforts towards understanding how to manage soil microbial communities based on specific functions (P solubilization and mineralization), and selection of microorganisms as potential microbial inoculants (biofertilizers) especially in no-tillage crop managements.

Methodology

Soil samples were taken the rhizosphere of maize cultivars contrasting to P, maize hybrid efficient to P (RHT3060), inefficient (RHS26x1113), efficient inbred lines (RL3), inefficient line (RL22), growing in a conventionally managed low P acid soil ("Cerrado"soil), and maize rhizosphere samples at 8 Brazilian "Cerrado" sites: 1. HT3060 on no-tillage managed in Embrapa Maize and Sorghum (CNPMS), 2. Jardinópolis, São Paulo (JSP), 3. Londrina, Paraná (CNPS), 4. Morrinhos, Goiás (MGO), 5. Planaltina, Goiás (PGO), 6. Rio Verde/Grassland, Goiás (GRGO), 7. Rio Verde/ Sugar cane, Goiás (SCRGO), 8. Rio Verde/Swine manure, Goiás (SMRGO). The microorganisms isolated from rhizosphere of maize genotypes, inoculated on solid medium containing P inorganic insoluble forms $Ca_3(PO_4)_4$ (P-Ca), $AlPO_4$ (P-Al), and samples from the rhizosphere soil of 8 Brazilian sites of maize crops on no-tillage managed was inoculated in specific culture medium containing P organic insoluble fonts: Sodium phytate – Phytic Acid (P-phytate), Soybean lecitin (P-soybean). P-Ca, P-Al, P-phytate, P-soybean were autoclaved and added in Pikovskaya'agar medium at $5g.L^{-1}$, $3,5\ g.L^{-1}$, $10g\ L^{-1}$, and $15g\ L^{-1}$, respectively. Of the total 371 isolates, 45 microorganisms were selected, and were assayed in liquid culture to quantify P-solubilizing activity, pH solution, phosphatase production and P-Melich solubilizing activity. Each isolated was inoculated into three replicate flasks for 10 days. Three replicates control treatments were included containing medium only for the same P-insoluble sources. The 5mL sobrenadant aliquots were assayed for soluble P, pH solution, phosphatase activity (Freitas *et al.* , 1997 modified protocols; Tabatai and Bremmer, 1969). The 45 isolates most effective at solubilizing P-phytate, P-soybean, P-Ca, P-Al were identified based on nucleotide sequence data from the ITS region for fungal and 16S region for bacterial and actinomyces. PCR products were purified using QIAquick Gel Extraction kit and sequenced following using kit "Big Dye Terminator v3.1. Cycle Sequencing" on an ABI PRISM 3100 Genetic Analyzer. Nucleotide sequence data were compared with those on GenBank using the BlastN search (Altschul et al., 1997).

Results and Discussion

A total of 371 isolates of P-solubilizing microorganisms was recovered from the maize rhizosphere. Out of 36 isolated from P-Ca maize rhizosphere that solubilized more than 80mgP.L^{-1} (30% of total P) at 10 days, 14 (39%) were isolated from RL3. The most P-Al microorganisms solubilizing were found in maize genotypes rhizosphere inefficient to P (RL22 and RHS26x1113). However, the percentage of microorganisms most effective P-Al solubilizing activity in liquid medium (greater than 10mgP.L^{-1}, at 10 days) to RL3, RL22, RHT3060, RHS26x1113 was 54%, 22%, 50%, 35%, respectively. The total number of P-soybean-solubilizing isolates was uniformly distributed among samples, but in MGO samples, before soybean no-tillage soil samples, the percentage of effective isolates (level of P solubilization greater than 15mg.L^{-1}) was greatest (32%). The highest level of inorganic P solubilization was by the isolates from P-Ca medium, especially the actinomyces and bacterial, and the lowest level occurred to P-Al solubilizing microorganisms. P-Al microorganisms isolated were mainly fungal. It is possible that 10 days growing time might have been insufficient for P solubilization by fungi that have a slowly growth rate in culture solution. Among the organic phosphates sources tested, P-phytate supported highest solubilizing activity for the isolates examined, especially fungi, also presents in major number. In P-soybean medium, the number of actinomyces isolates found was highest, but the greatest solubilization was observed to fungi. The molecular characterization shown that close matches were found between the 16SrDNA sequences of bacterial and actinomyces, rDNA-ITS of fungi of the 45 screened isolates investigated in this study and existing entries in the GenBank database. Among the various isolates screened, B5, isolated from L3 rhizosphere (efficient to P genotype), was identified as uncultured *Bulkoderia sp* and was the most efficient to mobilize P from P-Ca source culture solution, solubilizing 70% of total P. Some species of *Bulkoderia* has been found to fixing N_2 and to solubilize phosphorus. The most effective P-Ca –solubilizing fungi (F14) was identified as *Penicillium pinophilum,* mobilizing 65% of total P. This fungi was isolated from rhizosphere of efficient maize genotype under conventional tillage, HT3060. Among actinomyces screened, P-Ca solubilization was greatest to *Streptomyces* collinus (A26), isolated also from rhizosphere of HT3060 efficient to P genotype, under conventional tillage. In solution culture containing organic P source as soybean lecitin the most effective isolate, solubilizing 22% of P was the A65 (*Kitasatosporia paracochleatus*) isolated from rhizosphere of HT3060 efficient to P genotype cropped in no-tillage management.

Conclusions

While comparing the relative efficiency of phosphate solubilizing organisms using different insoluble P sources, it was observed that, P solubilization depended on the nature of P source and the organism. P efficiency in these cultivars may be related to the potential to enhance microbial development colonization and symbiosis of solubilizing microorganisms. The isolated B5, from L3 line efficient to P, was the most effective, mobilizing 70% P, and was identified as "uncultured *Burkholderia sp*", a nitrogen fixing bacteria genera.

References

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