Phosphate Solubilizing Microorganisms Associated With The Rhizosphere Of Maize Grown In Brazilian "Cerrado", Under Conventional Tillage And No-Tillage Managements

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Introduction

Phosphorus is an essential element for plant development and is present in the soils in forms highly insoluble and unavailable to plants especially in acid soils (Marschner, 1995; Wakelin et al., 2004). The accumulated insoluble P, like total soil P, occurs in either organic or inorganic forms. 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, economica 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 fungi as potential microbial inoculants (biofertilizers) especially in no-tillage crop managements.

Methodology

Soil samples were taken from the rhizosphere of 4 maize cultivars contrasting to P: RHT3060 - maize hybrid efficient to P, RHS26x1113 - inefficient, RL3 - efficient inbred lines, RL22 - inefficient line. These genotypes were growing in a conventionally managed low P acid soil ("Cerrado"soil) of the breeding program at Embrapa Maize and Sorghum at Sete Lagoas, Minas Gerais, Brazil. Others 8 soil samples of the maize rhizosphere growing in Brazilian "Cerrado" sites under No-tillage managements were taken to organic P-solubilizing microorganisms analyzing: CNPMS – from Embrapa Maize and Sorghum , JSP - from Jardinópolis, São Paulo; CNPS- from Londrina, Paraná ; MGO - from Morrinhos, Goiás ; PGO - from Planaltina, Goiás; GRGO - from Rio Verde, Goiás (maize and grassland); SCRGO- Rio Verde, Goiás (maize and sugarcane); SMRGO - Rio Verde, Goiás (maize under swine manure). 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 were 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, solution pH, 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. After 10 days incubation, the cultures were centrifuged and filtrate. The 5 mL supernatant aliquots were assayed for soluble P, solution pH, 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 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

Phosphorus solubilizing microorganisms were recovered from the maize rhizosphere in a total of 371 isolates (Table 1). Thirty six isolates from P-Ca maize rhizosphere solubilized more than 80 mg P.L^{-1} (30% of total P), at 10 days, of these, 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 10 mg P.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 the 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. Bacterial and actinomyces 16S rDNA and fungi rDNA-ITS sequences matched closely 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, the greatest P-Ca solubilization was *Streptomyces* (A26), isolated also from rhizosphere of HT3060 an efficient 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 an efficient to P genotype cultivated in no-tillage management.

Table 1. Description of samples from maize rhizosphere and soils in which maize plants were grown; number of microorganisms (fungi, bacteria, actinomyces) isolated from conventional tillage soil samples in solid medium containing P inorganic insoluble fonts, calcium phosphate (P-Ca), aluminum phosphate (P-Al), and from no-tillage managed soils samples of solid medium containing P organic insoluble fonts, Sodium phytate (P-phytate), Soybean lecitin (P-soybean)

Sample	Soil (Oxisol)	City/State[a]	Manage/ Crop[b]	Total P inorganic-solubilizing isolated[c]		Total P organic-solubilizing isolated[c]	
				P-Ca	P-Al	P-phytate	P-soybean
RL3	Red latosol	Sete Lagoas/MG	CT/Maize	34 (14)	13 (7)	-	-
RL22	Red latosol	Sete Lagoas/MG	CT/Maize	23 (6)	18 (4)	-	-
RHT3060	Red latosol	Sete Lagoas/MG	CT/Maize	28 (9)	8 (4)	-	-
RHS26x1113	Red latosol	Sete Lagoas/MG	CT/Maize	21 (7)	17(6)	-	-
CNPMS	Red latosol	Sete Lagoas/MG	NT/Maize HT3060	-	-	2 (1)	21 (6)
JSP	Dusky Red latosol	Jardinópolis/SP	NT/Maize	-	-	6 (0)	25 (3)
CNPS	Dusky Red latosol	Londrina/PR	NT/Maize	-	-	3 (1)	10 (0)
MGO	Red latosol	Morrinhos/GO	NT/Maize (Soybean)	-	-	9 (3)	22 (7)
PGO	Red latosol	Planaltina/GO	NT/Maize (Grassland/ Soybean)	-	-	9 (1)	20 (0)
GRGO	Dusky Red Latosol	Rio Verde/GO	NT/Maize (Grassland)	-	-	13 (3)	15 (0)
SCRGO	Dusky Red Latosol	Rio Verde/GO	NT/Maize (Sugar Cane)	-	-	14 (10)	11 (3)
SMRGO	Dusky Red Latosol	Rio Verde/GO	SM/NT/ Maize	-	-	7 (0)	22 (5)
			Totals	**106**	**56**	**63**	**146**

[a] States acronym: MG, Minas Gerais; SP, São Paulo; GO, Goiás

[b] Manures Types: CT, Conventional Tillage; NT, No-Tillage; SM, Swine Manure, and plant type from under during which the soil was sampled and before (in parentheses).

[c] Total number of isolated and transferred onto solid medium containing P inorganic e organic insoluble fonts and the number of P solubilizing activity grown by the screened isolated in liquid medium (in parentheses), 10 days, that shown level of P solubilization greater than 80mgP. $L^{-1}$to P-Ca, 10mg.L^{-1} to P-Al, 10 mg.L^{-1} to P-phytate, 15 mg.L^{-1} to P-Soybean.

Conclusions

- ✓ While comparing the relative efficiency of phosphate solubilizing organisms using different insoluble P sources, it was observed that P solubilization depends 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.

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