Note

Solubilisation of inorganic phosphates by inoculant strains from tropical legumes

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ABSTRACT: Microbial solubilisation of low soluble inorganic phosphates is an important process contributing for the phosphorus available to plants in tropical soils. This study evaluates the ability of inoculant strains for tropical legumes to solubilise inorganic phosphates of low solubility that are found in tropical soils. Seven strains of Leguminosae nodulating bacteria (LNB) were compared with one another and with a non-nodulating positive control, *Burkholderia cepacia* (LMG 1222^T). Four of the strains are used as inoculants for cowpeas (*Vigna unguiculata*) (*Bradyrhizobium* sp. UFLA 03-84; *Bradyrhizobium elkani* INPA 03-11B and *Bradyrhizobium japonicum* BR3267) or for common beans (*Phaseolus vulgaris*) (*Rhizobium tropici* CIAT 899^T). *Rhizobium etli* UFLA 02-100 and *Rhizobium leguminosarum* 316C10a are also efficient nodulators of beans and *Cupriavidus taiwanensis* LMG 19424^T nodulates on *Mimosa pudica*. Two experiments, with solid and liquid media, were performed to determine whether the strains were able to solubilise $CaHPO_4$, $Al(H_2PO_4)_3$ or $FePO_4.2H_2O$. On solid GELP medium none of the strains dissolved $FePO_4.2H_2O$, but LMG 1222, UFLA 03-84 and CIAT 899 solubilised $CaHPO_4$ particularly well. These strains, along with LMG 19424 and BR 3267, were also able to increase the solubility of $Al(H_2PO_4)_3$. In liquid GELP medium, LMG 1222 solubilised all phosphate sources, but no legume nodulating strain could increase the solubility of $Al(H_2PO_4)_3$. The strains CIAT 899 and UFLA 02-100 were the only legume nodulating bacteria able to solubilise the other phosphate sources in liquid media, dissolving both $CaHPO_4$ and $FePO_4.2H_2O$. There was a negative correlation between the pH of the culture medium and the concentration of soluble phosphate when the phosphorus source was $CaHPO_4$ or $FePO_4.2H_2O$. The contribution of these strains to increasing the phosphorus nutrition of legumes and non-legume plant species should be investigated further by *in vivo* experiments.
Keywords: *Rhizobium*, *Bradyrhizobium*, *Cupriavidus*, phosphate-solubilising bacteria, nitrogen-fixing bacteria

Introduction

In tropical soils, the total phosphorus content is high; however, the content available to plants is very low, approximately 2 mg kg^{-1} extracted by Mehlich-1 (Lopes, 1989). This concentration is limiting to plant productivity, and it occurs because the majority of the applied phosphorus is absorbed on the surface of abundant minerals in these soils, such as iron and aluminium oxides, hydroxides and oxy-hydroxides. This is also due to the precipitation of phosphorus with Fe^{3+} and Al^{3+} ions in acid soils and with Ca^{2+} ions in alkaline soils (Tan, 1993). The reversibility of these non-labile forms of phosphorus is low and therefore less available to plants.

Among the alternatives that enable improvements in the efficient use of insoluble inorganic phosphates, we emphasise the action of microorganisms able to solubilise inorganic low soluble phosphates (Gerretsen, 1948; Sylvester-Bradley et al., 1982). The majority of studies looking at phosphate-solubilising microrganisms involve studies with fungi and free-living bacteria in the soil (Sousa et al., 2008; Bojinova et al., 2008; Linu et al., 2009; Ali et al., 2010).

This ability to solubilise is found even among leguminosae nodulating bacteria (LNB), such as *Rhizobium*, *Bradyrhizobium* and *Mesorhizobium* (Halder et al., 1990; Peix et al., 2001) and other non specified LNB species (Hara and Oliveira, 2004; 2005). *Rhizobium* together with *Pseudomonas* and *Bacillus,* are among the bacteria with the highest solubilisation potential (Rodriguez and Fraga, 1999). Thus, besides the ability to fix atmospheric nitrogen, legume-nodulating bacteria can also contribute to the growth of plants through solubilising inorganic phosphates of low solubility. Therefore, the ability of legume-nodulating microorganisms to solubilise these inorganic phosphates may also be considered when selecting strains with a high potential to fix N_2 so that they can be used not only to fix nitrogen but also to increase the availability of phosphorus to plants. But until now, few microorganisms that are symbiotic with legumes used for human consumption in the tropics, such as cowpeas and the common bean, have been evaluated for this ability and no information is available for those used as inoculants. Thus, the aim of this study was to assess the ability of type and inoculant strains for legume crops to solubilise inorganic phosphates that are found in tropical soils.

Materials and Methods

The experiments were performed in Lavras, state of Minas Gerais, Brazil (21º13'36.5" S; 44º58'44.23" W). The treatments used a non-legume-nodulating strain of *Burkholderia cepacia* (LMG 1222^T) as a positive control and the following seven strains of legume-nodulating bacteria: UFLA 03-84 (*Bradyrhizobium* sp.), INPA 03-11B (*Bradyrhizobium elkani*) and BR 3267 (*Bradyrhizobium japonicum*), all of which are approved by Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) as inoculants for cowpeas (*Vigna* unguiculata L. Walp.) (Martins et al., 2003; Lacerda et al., 2004; Soares et al., 2006); LMG 19424^T (*Cupriavidus taiwanensis*) isolated from "sensitive plant" (*Mimosa pudica*) nodules (Chen et al., 2001); CIAT 899^T (*Rhizobium tropici*), approved by MAPA as an inoculant for common beans (*Phaseolus vulgaris* L.); UFLA 02-100 (*Rhizobium etli*), a strain that currently has a good performance as inoculants for common beans (Soares et al., 2006); 316C10a (*Rhizobium leguminosarum* bv *phaseoli*). More information about the strains is found in Table 1.

Two experiments, one on solid and the other in liquid GELP medium (Sylvester-Bradley et al., 1982) containing (g L^{-1}) (glucose, 10.0; peptone, 5.0; yeast extract, 0.05; soil extract, 100 mL (The filtered supernatant of 1 kg soil in 1 L distilled water, autoclaved and allowed to stand 48 h); $MgSO_4$ (10 %), 2 mL; $CaCl_2$ (1 %), 2 mL; NaCl (10 %), 1 mL; micronutrient solution, 2 mL ($Ca_2MoO_4.2H_2O$, 0.200; $MnSO_4.H_2O$, 0.235; H_3BO_3, 0.280; $CuSO_4.5H_2O$, 0.008; $ZnSO_4.7H_2O$, 0.024, dissolved in 200 mL distilled water); Fe-EDTA (1.64 %), 4 mL; yeast extract, 0.05 and agar 15.0 when solid medium, were conducted to determine whether the strains were able to solubilise calcium ($CaHPO_4$), aluminium ($Al(H_2PO_4)_3$) and iron ($FePO_4.2H_2O$) inorganic phosphates. This growth medium was supplemented with one of three sources of phosphorus ($CaHPO_4$, $Al(H_2PO_4)_3$ and $FePO_4.2H_2O$) at a phosphorus concentration of 890 and 100 mg L^{-1} for the solid and liquid medium, respectively. $CaHPO_4$ was generated by adding 50 mL of a 10 % K_2HPO_4 solution and 100 mL of a 10 % $CaCl_2$ solution (autoclaved separately) to complete 1,000 mL culture medium to produce a precipitate of insoluble inorganic phosphate. The media containing aluminium or iron phosphates were generated by adding, prior to autoclaving, the reagents $Al(H_2PO_4)_3$ and $FePO_4.2H_2O$, previously ground and passed through a 0.062 mm mesh sieve. In the treatments containing $Al(H_2PO_4)_3$, the pH was adjusted to 4.5 while in the treatments containing $CaHPO_4$ and $FePO_4.2H_2O$, the pH was adjusted to 7.0. The precipitation of the low solubility phosphate in the three different media was verified by a "milky' appearance. We took care to mix the phosphate in the medium throughout the plate by agitating it carefully, just after pouring the medium, while it was still liquid at a temperature around 60 ºC.

To produce and standardize the inocula, the strains were inoculated into liquid 79 medium (Fred and Waksman, 1928) containing (g L^{-1}): K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; mannitol, 10.0 and yeast extract, 0.4; at pH 6.8. The strains were incubated under agitation (100 rpm) at room temperature under aerobic conditions. Using a spectrophotometer at a wavelength of 560 nm, readings were taken periodically until the strains reached an OD_{560} of 0.5, approximately equal to the 10^8 cells per mL (a 0.85 % saline solution was used to adjust cells to the desired density when the OD_{560} exceeded 0.5). For the evaluations on solid GELP media with low soluble phosphate treatment, four 20 µL aliquots of each culture (strains) with 0.5 OD_{560} were inoculated per 9.5 cm diameter Petri plate. The control treatment consisted of GELP medium containing the tested phosphate without inoculating any bacteria strains. The cultures on plates were incubated at 28 °C and the diameter of the solubilisation halo (translucent area around the colony) was measured using a digital calliper at the beginning of solubilisa-

Table 1 – Origin, identification and characteristics of the strains.

Strains	Origin	Identification	GenBank Access 16S-rDNA sequence	[5]Characteristics in medium "79"			
				Growth[1]	pH[2]	Color[3]	Diameter
							mm[4]
UFLA 03-84	Brazil	*Bradyrhizobium* sp. (Lacerda et al., 2004; Soares et al., 2006)	AF384136	S	alkaline	white	1 - 2
INPA 03-11B	Brazil	*Bradyrhizobium elkani* (Lacerda et al., 2004; Soares et al., 2006)	AF208510	S	alkaline	white	1
BR 3267	Brazil	*Bradyrhizobium japonicum (*Martins et al., 2003)	AY649439	I	alkaline	white	1 - 2
UFLA 02-100	Brazil	*Rhizobium etli* (Soares et al., 2006)	AY465886	F	neutral	white	> 2
CIAT 899	Colombia	*Rhizobium tropici* (Martinez-Romero et al., 1991)	M64405	F	acid	yellow	> 2
LMG 1222	United States	*Burkholderia cepacia* (Sinsabaugh and Howard (1975); Palleroni and Holmes (1981); Yabuuchi et al. (1992))	-----	F	acid	yellow	> 2
LMG 19424	Taiwan	*Cupriavidus taiwanensis* (Chen et al., 2001)	AF300324	F	alkaline	white	1
316C10a	Australia	*Rhizobium leguminosarum* (De Ley and Rassel, 1965)	-----	F	neutral	yellow	1

[1]Growth rate as time of appearance of isolated colonies: F = fast (2 to 3 days); I= intermediate (4 to 5 days); S= slow (6 to 10 days). [2]pH of the culture medium after growth. [3]Colony color. [4]Colony diameter. [5]Culture medium "79" (Fred and Waksman, 1928).

tion and at the end of the 15 day incubation period. From these measurements, the Solubilisation Index (SI) = halo diameter (mm) / colony diameter (mm) was calculated (Berraquero et al., 1976). Strains were classified as early when the solubilisation was initiated before the third day, late when solubilisation was initiated after the third day or non-solubilising when there was no visible solubilisation after fifteen days of evaluation. Based on the solubilisation index, strains were classified as low (SI < 2.00), intermediate (2.00 ≤ SI < 4.00) or high (SI ≥ 4.00) for their ability to solubilise. Berraquero et al. (1976) use "medium" instead "intermediate", we changed the term to avoid confusion with the term "medium" referring to the bacterial culture.

For liquid media, the evaluation was conducted by inoculating a 1 mL aliquot of the culture in liquid 79 medium, with OD_{560} of 0.5, into 50 mL GELP medium containing the low soluble inorganic phosphate in a 125 mL Erlenmeyer containing the previously mentioned sources of low soluble inorganic phosphate. These cultures were then incubated at 28 ºC for five days with an agitation of 130 rpm (centrifugal Hermle Z 323 K). At the end of this period, each sample was centrifuged (13,500 rpm for 5 min), the pH was determined and the levels of soluble phosphorus in the supernatant were quantified using the phosphomolybdate method (Murphy and Riley, 1962). The control treatment consisted of GELP medium containing the source of phosphate tested in the absence of bacteria. The ability of each strain to solubilise phosphorus was determined by taking the difference between the concentration of soluble phosphorus found in the inoculated culture medium and the concentration in the control treatment.

The experiments using liquid GELP media were designed as independent tests for each source of phosphate in a completely randomised design with three replications. The results were submitted to variance analysis using Sisvar 5.3 software (Ferreira, 2008) and the averages were compared using the Scott-Knott test at 5 %.

Results

All the strains were able to solubilise at least one of the low soluble inorganic phosphates in the solid GELP medium except for INPA 03-11B, which did not have a solubilisation halo on the three tested phosphate sources (Table 2). Strains LMG 1222, CIAT 899, BR 3267 and UFLA 03-84 were able to solubilise both $CaHPO_4$ and $Al(H_2PO_4)_3$, while the strain LMG 19424 was only able to solubilise $Al(H_2PO_4)_3$. None of the strains were able to solubilise $FePO_4.2H_2O$ in solid GELP medium. LMG 1222, UFLA 03-84 and CIAT 899 had an intermediate index of solubilisation in the medium with $CaHPO_4$ at the end of the experimental evaluation. On medium with $Al(H_2PO_4)_3$, solubilisation indices were low for the strains that were able to solubilise it. Therefore, the solubilisation indices for $CaHPO_4$ were higher than the indices for $Al(H_2PO_4)_3$.

All the strains produced early solubilisation in the $CaHPO_4$ medium except UFLA 02-100, which was classified as late. Although several strains did not have a solubilisation halo on the solid medium containing $CaHPO_4$ or $FePO_4.2H_2O$, some were able to grow on these media, especially on the $FePO_4.2H_2O$ medium (Table 2). This growth did not occur on the medium with $Al(H_2PO_4)_3$, since the only strains that grew on this medium were those that were able to solubilise it. The solubilisation by two strains on media with $CaHPO_4$ and $Al(H_2PO_4)_3$ and the growth without solubilisation on medium with $FePO_4.2H_2O$ can be seen in Figure 1.

The levels of soluble phosphorus in the non-inoculated controls, in the evaluation of solubilisation in liquid GELP medium, were 62.51 mg L^{-1} for $CaHPO_4$, 81.31 mg L^{-1} for $Al(H_2PO_4)_3$ and 20.93 mg L^{-1} for $FePO_4.2H_2O$ (Figure 2). Strain LMG 1222 was the only one able to increase the solubility of all the sources of inorganic phosphate, with the highest increase in solubility for $CaHPO_4$, followed by $FePO_4.2H_2O$ and $Al(H_2PO_4)_3$. Of the legume-nodulating

Table 2 – Solubilisation index for type strains and legume inoculants grown on solid GELP media with $CaHPO_4$ and $Al(H_2PO_4)_3$.

Strains	Solubilisation index (S.I.)			
	$CaHPO_4$		$Al(H_2PO_4)_3$	
	Initial (days)	End	Initial (days)	End
UFLA 03-84	1.27* (3)	2.12	1.18 (3)	1.22
INPA 03-11B	GNS	GNS	NG	NG
BR 3267	1.78 (3)	1.78	1.22 (3)	1.20
UFLA 02-100	1.26 (6)	1.26	NG	NG
CIAT 899	2.01 (3)	2.09	1.09 (3)	1.15
LMG 1222	2.51 (3)	3.06	1.20 (3)	1.28
LMG 19424	GNS	GNS	1.21 (3)	1.24
316C10a	1.06 (3)	1.12	NG	NG

*S.I = diameter halo (mm) / diameter colony (mm); Initial = reading performed once the start of solubilisation was detected; End = reading performed after 15 days of incubation; GNS = Grew and did not solubilise; NG = No growth.

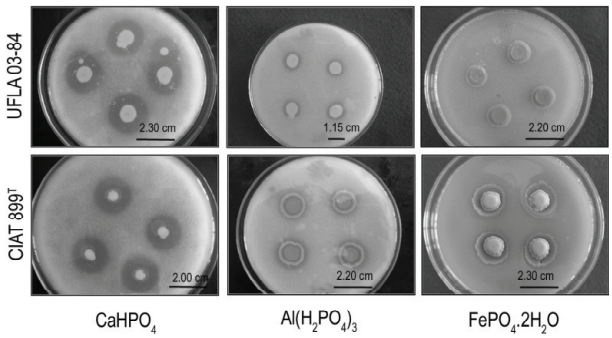

Figure 1 – Solubilisation halo of the low soluble inorganic phosphates ($CaHPO_4$ and $Al(H_2PO_4)_3$) and the bacterial growth without a solubilisation halo ($FePO_4.2H_2O$) on solid GELP media by nitrogen-fixing bacteria that nodulate legumes. Legume inoculants for cowpeas and common beans (UFLA 03-84 and CIAT 899) incubated at 28 °C for 15 days.

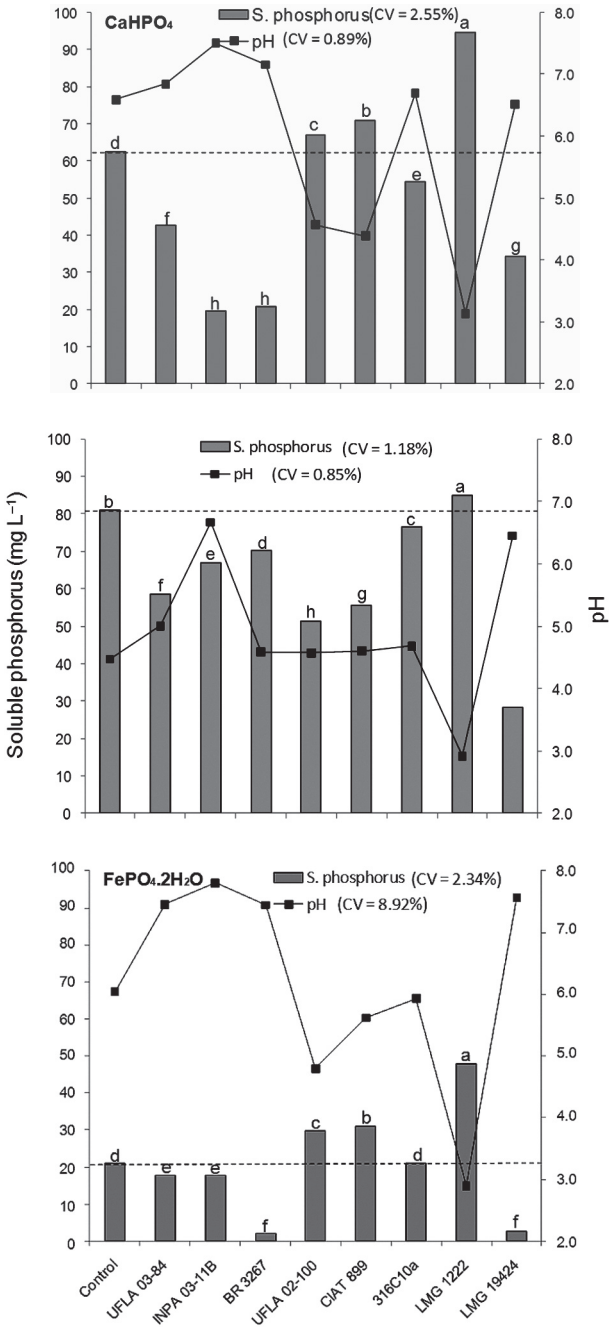

Figure 2 – Solubilisation and pH in liquid GELP medium after incubating the type and legume inoculants strains in the presence of $CaHPO_4$, $Al(H_2PO_4)_3$ and $FePO_4.2H_2O$. Means followed by the same letter did not differ (Scott-Knott test, $p \leq 0.05$). The dashed line represents the value of soluble phosphorus found in the control media.

strains able to solubilise phosphate on solid GELP media, only strains CIAT 899 and UFLA 02-100 were able to solubilise $CaHPO_4$ and $FePO_4.2H_2O$ in liquid GELP medium (Figure 2). Both strains solubilised higher concentrations of phosphate from $FePO_4.2H_2O$ compared to $CaHPO_4$. However, most of the remaining strains in addition to not having solubilisation ability in any of three sources of phosphates in liquid medium actually reduced what was available initially, compared with the uninoculated control. This may be due to production of substances of microbial metabolism and increase of medium pH thereby causing precipitation of soluble phosphorus and also due to consumption by the bacteria themselves, especially in the media with $CaHPO_4$ and Fe $PO_4.2H_2O$ (Figure 2).

All the strains that solubilised phosphate in liquid media reduced the pH of the media compared to the non-inoculated control, regardless of the source of phosphate. The strains that did not reduce the pH of the media were unable to solubilise phosphate. There was a negative correlation (Figure 3) between the amount of soluble phosphorus and the pH of culture media for $CaHPO_4$ ($r = -0.89$; $p \leq 0.05$) and $FePO_4.2H_2O$ ($r = -0.90$; $p \leq 0.05$); while this relationship was not observed for $Al(H_2PO_4)_3$ (data not shown). The *Bradyrhizobium* strains, UFLA 03-84, INPA 03-11B and BR 3267, that did not solubilise phosphate in liquid media, increased the pH of the culture media compared to the control for all the sources of phosphate. This is a typical characteristic for the genus, and the fast-growing *Cupriavidus taiwanensis* strain LMG 19424 did the same for $Al(H_2PO_4)_3$ and $FePO_4.2H_2O$ but did not alter the pH of GELP medium with $CaHPO_4$.

Discussion

The solubilisation index of strains varied from low to intermediate on medium with $CaHPO_4$. A study conducted with isolates and mutants of *Azotobacter chroococcum* inoculated into JM medium containing (g L^{-1}) [(sucrose, 20.0; K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.5; NaCl, 0.2; $CaCl_2$, 0.1; $FeSO_4.7H_2O$, 0.1; Na_2MoO_4, 0.005; agar, 20.0; pH 7.0)] and Pikovskaya medium (PVK) containing (g L^{-1}) [(glucose, 10.0; $(NH_4)_2SO_4$,

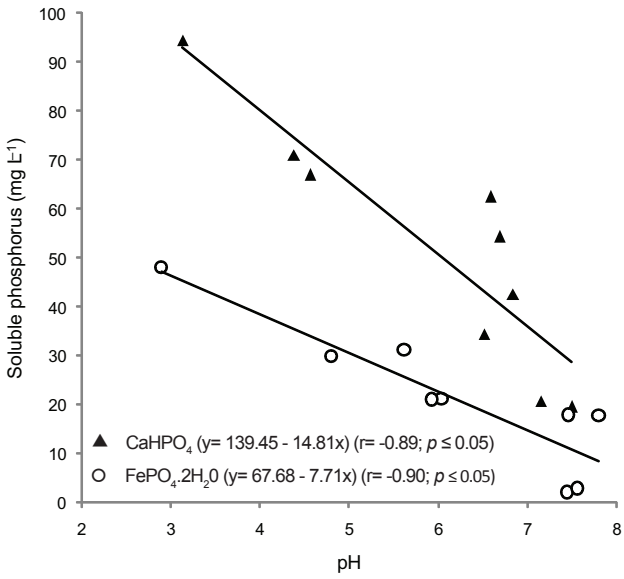

Figure 3 – Pearson correlation between the pH of the liquid GELP medium and the level of soluble phosphorus for the $CaHPO_4$ and $FePO_4.2H_2O$ sources after a five day incubation period with the type and legume inoculants strains (n = 9).

0.5; NaCl, 0.2; $MgCl_2$, 0.1; KCl, 0.2; yeast extract, 0.5; traces of $MnSO_4$ and $FeSO_4$; agar, 25.0; pH 7.0)] both with 2 % tricalcium phosphate that were incubated at 30 °C for seven days, also presented an intermediate solubilisation index in the range of 1.61 and 2.13, respectively (Kumar and Narula, 1999). For the liquid media, the same authors working with free-living nitrogen fixing *A. chroococcum* in JM medium with 2 % tricalcium phosphate and JM medium with 1 % Mussoorie natural phosphate observed, respectively, 1.43 and 0.20 mg L^{-1} of available phosphorus compared to the non-inoculated control. These values were lower than the increase in solubility obtained for strains CIAT 899, UFLA 02-100 and LMG 1222 solubilising $CaHPO_4$ in the liquid GELP medium, which could be due to a higher solubilisation potential of the legume-nodulating strains. Furthermore, the strains LMG 1222, CIAT 899, BR 3267 and UFLA 03-84 were able to solubilise $CaHPO_4$ (pH 7.0) and $Al(H_2PO_4)_3$ (pH 4.5) on solid GELP medium, which may extend the range of activity of these strains in soils with different pH values.

Although none of the strains were able to solubilise $FePO_4.2H_2O$ on solid GELP medium, strains CIAT 899, UFLA 02-100 and LMG 1222 were able to increase solubility of $FePO_4.2H_2O$ in liquid GELP medium. It is possible that a smaller amount of solubilisation may have occurred on the solid medium without the formation of the characteristic halo around the colony as observed by Perez et al. (2007). As for the other two sources of phosphate, most strains were only able to solubilise them on the solid GELP media, which may be related to the production of exopolysaccharides by the bacteria since these compounds produced on solid media remain concentrated locally and may function to solubilise phosphates in conjunction with organic acids (Yi et al., 2008). On the other hand, in the liquid GELP media, the exopolysaccharides may be dispersed, decreasing their activity as a solubilising mechanism.

The highest solubilisation of $Ca_3(PO_4)_2$ followed by $FePO_4$ and $AlPO_4$ was observed in liquid PVK medium by bacteria from rhizosphere of green onions (*Allium fistulosum* L.), chilli peppers (*Capsicum annuum* L.), sesame (*Sesamum indicum* L .) and rice (*Oryza sativa* L.) (Chung et al., 2005). Other authors showed higher solubilisation for $Ca_3(PO_4)_2$ followed by $AlPO_4$ and $FePO_4$ by *Bacillus* sp., *Streptomyces thermophilus* and *Aspergillus fumigatus* in the same culture medium with the same sources of phosphate (Chang and Yang, 2009). These results differ from those found in our study, since phosphate solubility was increased by two legume-nodulating strains at least as much on $FePO_4$ as on $CaHPO_4$ in liquid GELP medium. This may be interpreted as a positive characteristic, since $FePO_4.2H_2O$ is one of the most predominant forms of phosphate in tropical and sub-tropical soils.

Strains of *Rhizobium* sp. isolated from *Sesbania cannabina* and *Bradyrhizobium* sp. isolated from *Crotalaria juncea* grown for three days at 27 ºC in medium 79 supplemented with 0.2 % tricalcium phosphate produced 79.0 and 5.0 mg L^{-1} of soluble phosphorus respectively (Daimon et al., 2006), showing that strains of *Rhizobium* have a greater ability to solubilise compared to strains of *Bradyrhizobium*. These results corroborate those found in this study for the liquid GELP medium, given that CIAT 899 (*Rhizobium tropici)* and UFLA 02-100 (*Rhizobium etli*) were the only nodule bacteria able to solubilise $CaHPO_4$ and they were also able to solubilise $FePO_4.2H_2O$. Other studies using strains of *Rhizobium* isolated from *Crotalaria* sp. inoculated into PVK medium and incubated for nine days found solubilisation indices ranging from 2.40 to 2.70 (Sridevi et al., 2007), which are considered intermediate values (Berraquero et al., 1976). For some species, the decrease in pH is an essential condition that allows the insoluble inorganic phosphates to be solubilised. These results may be related to the different metabolism of different species, since the genus *Rhizobium* usually produces an acidic reaction in medium 79 while the genus *Bradyrhizobium* commonly produces an alkaline reaction.

A significant negative correlation between the pH of the culture media and the levels of available phosphorus was observed when the source of phosphorus was $CaHPO_4$ or $FePO_4.2H_2O$. This may indicate a trend towards solubilisation only for the strains that are able to reduce the pH of the media. For example, strain LMG 1222 had the most notable reduction in the pH of the media for all the sources of phosphate and consequently had a greater ability to solubilise phosphate compared to the other strains. This decrease in pH is a basic principle in phosphate solubilisation and may be related to the production of organic acids and the release of protons (Sperber, 1958; Lin et al., 2006). Studies with free-living nitrogen-fixing bacteria (Kumar and Narula, 1999), rhizobia (Daimon et al., 2006; Sridevi et al., 2007) and free-living soil bacteria (Rajkumar and Freitas, 2008) also showed a negative correlation for phosphate solubilisation and pH.

In Brazil, studies with rhizobia from the Amazonian region have demonstrated the reduced ability to solubilise calcium and aluminium phosphates in solid medium after an 18 day incubation on a medium developed by Sylvester-Bradley et al. (1982) for calcium phosphate and on a medium with aluminium phosphate developed by the authors of this study (Hara and Oliveira 2004; 2005). Only one isolate had a high solubilisation index on a medium with calcium phosphate while the majority of the isolates had early solubilisation. For the time to initiate solubilisation, the results found by the authors mentioned above corroborate to those found in this study since only one isolate did not have an early solubilisation index.

Besides contributing to plant growth by making soluble phosphorus more available, the legume-nodulating strains that increased levels of soluble phosphate can improve the efficiency of biological nitrogen fixation, given that nodulated plants require more phosphorus than the plants that use only mineral nitrogen (Silva et al., 2006). Thus, low levels of phosphorus can affect symbiosis by decreasing the supply of photosynthates to the nodule, which reduces the rate of bacterial growth and the total population of legume-nodulating microorganisms (Moreira et al., 2010). This also suggests to us that the efficiency of nitrogen fixation by the strains approved as inoculants may be related to a greater ability to solubilise low soluble phosphates; however, no studies have

experimentally demonstrated this so far. The strong ability of *Burkholderia cepacia* to solubilise phosphates has already been demonstrated (Lin et al., 2006; Song et al., 2008); however, its management in soil is limited due to its high level of human pathogenicity.

Studies involving the solubilisation of iron phosphate have previously been conducted with free-living soil bacteria (Chung et al., 2005; Perez et al., 2007). Our results are novel in evaluating legume-nodulating bacteria for tropical crops and finding several strains that can solubilise this common phosphate source. Therefore, the strains tested in this study could be used for future *in planta* experiments to assess their ability to contribute to an increase in the availability of phosphorus to both legume and non-legume species

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