Preliminary Study on Phosphate Solubilization and K-releasing Abilities of *Rhizobium tropici* Martinez-Romero *et al.* Strains from Woody Legumes

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Abstract
Experiments were conducted on phosphate solubilization and K-releasing abilities of standard strain CGMCC 1.2540^T of *Rhizobium tropici* Martinez-Romero *et al*. (1991) and 22 strains of *Rhizobium tropici* Martinez-Romero *et al*. (1991) which were separated from woody legume plants in the Jianfengling Nature Reserve, Hainan Province. None of the tested strains have shown the ability to solubilize organic phosphate on Mongina organic culture medium, but have demonstrated K-releasing ability on K-releasing culture medium. 20 strains have the ability to solubilize inorganic phosphate of $Ca_3(PO_4)_2$. Different strains indicate great differences in phosphate solubilization and K-releasing abilities, particularly so in phosphate solubilization. The weakest ability of phosphate solubilization by the strain of Caf438 and the strongest by Caf341 increased the available phosphoru in the medium by 0.796 mg L^{-1} and 628.57 mg L^{-1} respectively, of which the difference is 788 times; The abilities of phosphate solubilization and K-releasing are irrelevant of hosts. The strains of *Rhizobium tropici* Martinez-Romero *et al*. (1991) on the same host show apparent differences on phosphate solubilization and K-releasing abilities. The strains of Caf336, Caf344 and Caf414 are provided with more powerful phosphate solubilization and K-releasing abilities.

Key Words
Woody legume, rhizobia, phosphate-solubilized halo, phosphate solubilization, content of available P, K-releasing ability, content of available K

Introduction

The symbiosis of rhizobia and legume plants is the strongest system in bio-fixation of nitrogen, of which the nitrogen fixed accounts for 65% of all the nitrogen fixed biologically (Danming *et al*., 2002; Zabran, 1999; Denarie and Roche, 1991). Moreover, the developed root system of woody legumes make them stronger in nitrogen fixation in comparison with grain legumes of soybean, peanuts, broad bean and others (80-210 kgN/ ha.a), which is 43-581 kgN / ha.a (Fuli and Zhengjia, 2000). The current studies on rhizobia are concentrated on the function of nitrogen fixation and their relationship with host plants, while the abilities of their phosphate solubilization and K-releasing are less dealt with. N, P and K are three main essential nutrition elements for plants. In China, the total content of P in soil is high, but of which 95% is not available in the forms of aluminnosilicate, phosphorite, etc. that is difficult to be used directly by plants. Soil is a natural reserve of potassium, the K content of the tilled soil layer is about 26,100 kg/hm^2, of which 90% exists in potassium feldspar, mica and other silicate minerals. Those are stable minerals that could not be used directly by plants, leading to the phenomenon of K rich but available K lacking in soil. Phosphate solubilizing microbe convert and make available P in soil and the study of P solubilization by bacteria mainly deals with silicate bacteria (Baogui and Bin, 2005; Jian *et al*., 2000; Fengding *et al*., 1997). But those microbes are found in a free state in soil, which hinders the conversion and use of unavailable P and K in soil, owing to the competition with indigenous microbes and their survival time. P solubilization and K releasing by rhizobia is seldom reported. Rhizobium and its symbiotic plant forms a specific structure – root nodule, which is endowed with a unique advantage in survival time and competibility compared with other bacteria. The screening of P solubilizing and K-releasing rhizobia strains has high academic, ecological and economic values.

Material and Methods

Bacterial strains

The 22 strains of Rhizobium tropici Martinez-Romero *et al.* (1991) were isolated from the root nodules of legume plants in the Jianfengling Nature Reserve, Hainan Province. The strains of Caf224, Caf225, Caf226 were isolated from Ormosia balansae Drake, Caf276, Caf278 and Caf279 were isolated from Desmodium triquetrum (Linn.) DC., Caf333, Caf334, Caf336 were isolated from Desmodium heterocarpon (L.) DC., Caf341, Caf344 were isolated from Indigofera suffruticosa Mill. Caf414, Caf415, Caf416 were isolated from Ormosia semicastrata Hance f. Litchifolia, and Caf436, Caf437, Caf438, Caf439, Caf440, Caf443, Caf444, and Caf446 were isolated from Acacia mangium Willd., while standard strain CGMCC 1.2540T was from cgmcc.

Media and methods

1. Bevel culture media: Rhizobia culture media-1 (Yuguang, 2007).
2. Selecting strains of inorganic phosphate-solubilized on PKO medium and solubilize organic P (lecithin) on Mongina organic culture medium. Checking up the phosphate-solubilized halo of the tested strains
3. Liquid culture medium (Chuanjin and Lin, 2002).
4. The inorganic phosphate culture medium： Glucose 10 g, $(NH_4)_2 SO_4$ 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, NaCl 0.3 g, KCl 0.3 g, $FeSO_4 \cdot 7H_2O$ 0.03 g, $MnSO_4 \cdot 7H_2O$ 0.03 g, $Ca_3(PO_4)$ 2 5 g, distilled water 1000 mL, pH 7.2, 110 ^oC Sterilization for 30 min.
5. The Organic phosphate culture medium: Glucose 10 g, $(NH_4)_2 SO_4$ 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, NaCl 0.3 g, KCl 0.3 g, $FeSO_4 \cdot 7H_2O$ 0.03 g, $MnSO_4 \cdot 7H_2O$ 0.03g, lecithin 2 g, $CaCO_3$ 5 g, distilled water 1000 mL, pH 7.2, 110 ^oC , Sterilization for 30 min.
6. Determining available P content of the culture solution used Mo anti-antimony colorimetry.
7. Ashby culture medium (Yuguang, 2007)
8. KH_2PO_4 0.2 g, $MgSO_4 7H_2O$ 0.2 g, NaCl 0.2 g, $CaCO_3$ 5.0 g mannite 10 g, $CaSO_4 2H_2O$ 0.1 g, distilled water 1.0L, pH7.0. Choose the colony with slime.
9. K-releasing culture medium (Institute of Soil Science, 1985)
10. Saccharose 5.0 g, Na_2HPO_4 2.0 g, $MgSO_4 \cdot 7H_2O$ 0.5g $FeCl_3$ 0.005 g, $CaCO_3$ 0.1 g, $KAlSi_3O_8$ 5 g, distilled water1.0 L. Determine available P content of the culture solution with Atomic Absorption Spectrophotometer Method.

Data processing

Data processing using Excel and Spss16.0

Result

Selecting the rhizobia strains of phosphate solubilization

Checking the phosphate-solubilized halo.On PKO inorganic medium flat, 20 strains have generated phosphate-solubilized halo except Caf226, Caf438 and CGMCC 1.2540T. The result shows that the 20 strains could solubilize nullification P ($Ca_3(PO_4)_2$). On Mongina organic culture medium, all of 23 tested strains show no phosphate-solubilized halo. Figure1 shows the phosphate-solubilized halo of Caf341 strain On PKO inorganic medium flat.

Figure 1. Phosphate-solubilized halo of Caf341 strain.

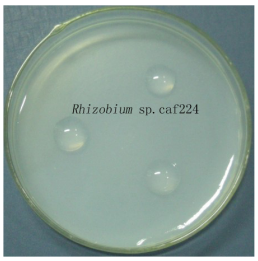

Figure 2. Caf224 colony on potassium medium culture.

The ability of diferent Rhizobia isolated at solubilizing inorganic phosphate

Table 1. The Ability of Diferent Rhizobia Isolated at Solubilizing Inorganic Phosphate.

Strains	Content of available P of the *Rhizobia* culture with $Ca_3(PO_4)_2$ as only P source	Strains	Content of available P of the *Rhizobia* culture with $Ca_3(PO_4)_2$ as only P source
Caf224	13.81**	Caf415	14.44**
Caf225	22.18**	Caf416	45.20**
Caf226	2.51	Caf436	585.06**
Caf276	528.56	Caf437	418.69**
Caf278	421.85	Caf438	1.86
Caf279	101.69**	Caf439	139.36**
Caf333	239.79**	Caf440	170.75**
Caf334	618.40**	Caf443	264.90**
Caf336	403.01**	Caf444	499.31**
Caf341	629.63**	Caf446	126.80**
Caf344	426.87**	CGMCC1.2 540T	1.89
Caf414	422.35**	ck	1.06

**level0.011

In Table 1, content of available P of 20 strains with phosphate-solubilized halo is higher than that of the control evidently. Different strains indicate great differences in phosphate solubilization. The weakest ability of phosphate solubilization by the strain of Caf438 and the strongest by Caf341 increased the available phosphate in the medium by 0.796 mg L^{-1} and 628.57 mg L^{-1} respectively, of which the difference is as great as over 788 times; The contents of available P in culture liquid of Caf341, Caf334, Caf436, Caf276, Caf444, Caf344, Caf414, Caf278, Caf437 and Caf336 are more than 400 mg L^{-1}.All of 23 strains are Rhizobium tropici Martinez-Romero *et al*. (1991) The result shows that different strains of same species have different abilities of phosphate solubilization.

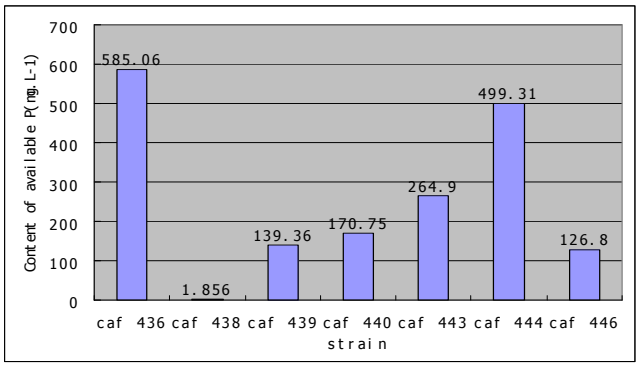

Figure3. Contents of available P of the *Rhizobia* culture (host: *Acacia mangium* Willd.)

The abilities of phosphate solubilization of strains which were isolated from the same host are different. Caf436 and Caf438 were isolated from the root nodule of *Acacia mangium Willd*, which have increased the available phosphate in the culture liquid by 0.796 mg L^{-1} and 584 mg L^{-1} respectively, of which the difference is as great as over 733 times.

Selection of strains with K-releasing ability

All the 23 tested strains could grow on Ashby culture medium and K-releasing culture medium. All of the colonies are smooth, transparent and with slime. This shows that all strains could utilize K of $KAlSi_3O_8$. See Figure 2.

Determination of K-releasing ability

The content of available potassium from the filtrates of culture were determined with Atomic Absorption Spectrophotometer Method (Table 2). All of the tested strains were apparently different in K-releasing ability from the control (F0.01=0.000<0.01), with a sharp contrast between Caf224 and Caf416 against the standard strain of CGMCC1.2540. Caf224, Caf416, Caf420, Caf278, Caf440, Caf341, Caf344, Caf414 and Caf415 of Rhizobium tropici Martinez-Romero *et al*. (1991) show apparent differences in on phosphate solubilization and K-releasing abilities, compared with the standard strain. And the ability of strains in K-releasing is not related with hosts.

Table 2. Available Potassium Contents in Different Rhizobia Cultures.

	Available potassium content （mg/L）					Available potassium content （mg/L）			
Strains	1	2	3	Average	Strains	1	2	3	Average
Caf224	8.272	7.735	8.248	8.272**	Caf415	7.434	7.634	7.908	7.659**
Caf225	7.256	6.296	6.729	7.256**	Caf416	8.39	7.998	7.692	8.027**
Caf226	7.706	7.241	7.882	7.610**	Caf436	6.422	6.849	6.452	6.574**
Caf276	6.769	6.293	7.315	6.792**	Caf437	6.849	7.185	6.786	6.940**
Caf278	6.964	8.211	6.704	7.293**	Caf438	7.288	7.066	6.668	7.007**
Caf279	6.977	6.804	6.720	6.834**	Caf439	7.034	6.855	6.609	6.833**
Caf333	6.964	7.750	6.789	7.168**	Caf440	7.24	7.537	7.21	7.329**
Caf334	7.111	6.886	7.495	7.164**	Caf443	6.693	7.806	6.709	7.069**
Caf336	7.794	7.512	7.446	7.584**	Caf444	6.840	6.701	6.673	6.738**
Caf341	7.249	7.834	7.091	7.391**	Caf446	6.764	6.450	7.969	7.061**
Caf344	7.763	7.534	7.153	7.483**	CGMCC1.2540^T	6.783	8.285	6.606	7.225**
Caf414	8.277	7.762	7.535	7.858**	Check	4.791	5.263	5.470	5.175

**level0.01

The correlation of abilities on phosphate solubilization and potassium releasing

Table 3. The Correlation of Abilities on P Solubilization and K Releasing.

		Content of available K	Content of available P
VAR00002	Pearson Correlation	1	-.007
	Sig. (2-tailed)		.954
	N	72	72
VAR00003	Pearson Correlation	-.007	1
	Sig. (2-tailed)	.954	
	N	72	72

It is seen from Table 3, the abilities of strains on phosphate solubilization and K–releasing are irrelevant in between. The result shows that phosphate solubilization and K–releasing are effected by different mechanisms.

Results and Discussion

1 The 22 strains which were separated from woody legumes in the Jianfengling Nature Reserve, Hainan Province have the ability to release K, and of which 20 have the ability to solubilize inorganic phosphate of $Ca_3(PO_4)_2$. The strains with strong P solubilization ability are Caf341, Caf334, Caf436, Caf276, Caf444, Caf344, Caf414, Caf278, Caf437 and Caf336.

2 Different strains demonstrate great differences in the abilities to convert P and K, particularly so in the conversion of P. The weakest ability of phosphate solubilization by the strain of Caf438 and the strongest by Caf341 increased the available phosphate in the medium by 0.796 mg L^{-1} and 628.57 mg L^{-1} respectively, of which the difference is as great as over 788 times.

3 The abilities of phosphate solubilization and K-releasing are irrelevant of hosts. The strains of Caf436, Caf437, Caf438, Caf439, Caf440, Caf443, Caf444 and Caf446 were all on the same host of *Acacia mangium Willd*, which, however, present a sharp contrast in between in the contents of available P and fast K.

4 Different strains of the same species indicate great differences in phosphate solubilization and K-releasing abilities. All the strains tested are of *Rhizobium tropici* Martinez-Romero *et al*. (1991) but different strains show a great contrast of available P and fast K in the culture.

5 The abilities of rhizobia in P solubilization and K–releasing are not correlated, which indicates different mechanisms for P solubilization and K–releasing, awaiting further study.

6 None of the rhizobia of 26 strains tested in the experiment is able to solubilize the lecithin of organic phosphate. Whether the rhizobia can solubilize other organic phosphates calls for further experiment.

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