

Phosphate-solubilizing microbes in rhizosphere soils of 19 weeds in southeastern China*

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Abstract: Low phosphorus (P) availability is one of the most important factors limiting plant growth in red soils across southeastern China. Many non-symbiotic microorganisms in rhizosphere can enhance P solubility, but little is known about the magnitude of their phosphorus-solubilizing ability (PSA) and the difference in phosphorus-solubilizing microorganisms (PSM) among plant species. The number of phosphorus-solubilizing microorganisms and their PSA in rhizosphere soils of 19 weed species in a citrus orchard on red soil at Changshan, Zhejiang, China, were investigated. Inorganic P (powdered phosphate rock, PR) and organic P (lecithin, OP) were respectively used as the sole P-source to examine the PSA of isolated microbes. The PS actinomycetes community varied greatly among the different weed rhizospheres while the PS fungus community showed to be most stable to the weed rhizosphere. The highest number of PR-PS and OP-PS bacteria was found in rhizosphere soil of *Mollugo pentaphyll*, and the highest number of PR-PS and OP-PS actinomycetes was found in rhizosphere soil of *Polygonum lapathi-folium*. The highest number of PR-PS fungi was found in *Erigeron annuus* and *Mollugo pentaphyll* rhizosphere soil, and the highest number of OP-PS fungi was found in rhizosphere soil of *Mazus stachydi-folius*. *Mazus stachydi-folius* showed the strongest PR-PS ability (6340.75 μ g) while *Eragrostis pilosa* showed the strongest OP-PS ability (1301.84 μ g). The PR-PS ability and OP-PS ability of *Mollugo pentaphyll* was 4432.87 μ g and 1122.05 μ g respectively. A significant correlation between the number of PR-PSM and OP-PSM was found. Significant correlation was only found between the PR-PS fungi number and its PSA ($r = 0.75$, $P < 0.05$) and between the number of OP-PS fungi and its PSA ($r = 0.87$, $P < 0.01$). It indicated that plant species had significant influence on components of the non-symbiotic PSM community and their activity in its rhizosphere soil. Fungi play a leading role in phosphorus solubilization in weed rhizosphere. It suggested that weed conservation could benefit soil microbe development in agroecosystems, especially in the initial stage of agroecosystem development because there is less organic carbon in bare soil. The results suggested that weed conservation could increase PSA of PSM.

Key words: Weed rhizosphere, Phosphorus-solubilizing microbes (PSM), Phosphorus solubilizing abilities (PSA)

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INTRODUCTION

Only a small proportion of the total phosphorus (P) in soil is available to plants because of the strong chemical fixation of P in soils. The remainder, excluding organically bound P, is in chemical forms that are, at best, only very slightly soluble (Stewart et al., 1980). Many microorganisms in soil can solubilize unavailable forms of organic or inorganic-bound P (Curl,

1986; Kucey, 1983). These PSM include bacteria, fungi and actinomycetes and naturally, the range of PSA within such a heterogeneous group is very wide. It has been demonstrated that these PSM are present in soil in different numbers. A large proportion of the PS bacteria population is found in plant rhizospheres and the number of PS bacteria in soil is influenced more by soil type and manner of its cultivation than by the physical composition or content of humus and N or P in

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soil.

Red soils (equivalent to Ultisols and Oxisols in U.S. soil taxonomy) account for 22% of the total surface land and support 43% of the population in China (Zhao et al., 1994), where mountainous and hilly areas account for about 79% of its land. Along with the utilization of slope land resources for agriculture, primary plants were destroyed and soil erosion became serious. Soil erosion, which leads nutrient loss and lower microbial activities, results in many problems to agricultural development in this area. Low availability of both indigenous and applied P to plants under such soil conditions requires annual application of large amount of P fertilizers for sustainable high yield of crops. But most of the applied phosphorus accumulates on the fine particles readily transported to surface waters through runoff, especially in hilly regions (He et al., 1995). In China, 70% of lakes and 50% of rivers are currently endangered by eutrophication, considered to be mainly due to P transportation from arable lands (Sun, 1995). Therefore, more consideration is now given to the exploitation of chemical-bound P in the red soils and the prevention of soil loss in the exploitation of the resources of mountainous and hilly areas (Wang et al., 2001).

Weeding by mechanical and chemical methods, the main methods for controlling weeds in the utilized hilly land in red soil area, usually cause soil erosion and adversely affect soil microbial activities. Therefore, suitable weed management is necessary to achieve high yield of crops and at the same time control soil erosion. Studies showed that keeping some weed species appropriately in a fruit orchard could conserve soil with little negative effect on cultivated plants in uplands (Chen et al., 1999a; Chen et al., 1999b; Chen et al., 2000). Appropriate weed maintenance also contributes to long term yield by increasing soil fertility (Weiner, 1990). But there are few reports on the influence of weed species on community components and activities of soil microorganisms, especially PSM.

This study aims to examine the community of PSM and its PSA, and quantify the PSA of PSM in different weed rhizosphere, and also aims to determine if there is any difference in PSA of microbes at the community levels among weed species in order to apply the knowledge

gained to implement weed diversity conservation.

MATERIAL AND METHODS

1. Site description

The study was carried out in a newly developed 150 ha orchard in typical red soil hilly region located at Changshan County (28° 54' N, 118° 30' E) in southwestern Zhejiang Province, China. Citrus (*Castanea mollissima* Blume) were planted in 1997 at density of 5.0 m × 5.0 m and covered only about 10% of the land area. The dominant weed species of naturally secondary vegetation in the site belonged to families of Poaceae, Leguminosae, Asteraceae, Polygonaceae, Lamiaceae, Euphorbiaceae, Vitaceae, Molluginaceae, Ranunculaceae, Primulaceae and Barasssicaceae.

2. Sampling technique

Nineteen main weed species were selected from the site with priority according to the abundance of weed species. Thirty weed plants of each species and its rhizosphere soil were randomly sampled from the orchard at its well-grown season around the year. The complete root system attached with cylindrical soil bulk with radius of 15 cm was sampled, bagged by polyethylene bag, carried back to laboratory, and stored at 4 °C until processing.

3. Preparation of rhizosphere soil suspension

The soil adhering tightly to roots was defined as rhizosphere soil (Curl, 1986). To collect the rhizosphere soil, weed root samples were shaken by hand carefully to remove large soil particles. Then 10 g root sample with rhizosphere soil was put into a 250 ml flask containing 100 ml sterilized water and then shaken in a rotary shaker for 15 min. The prepared soil suspension was diluted $10^{-2} - 10^{-11}$ for determining the total PS bacteria, fungi and actinomycetes population. The root was removed from the flask then. The rhizosphere soil left in the flask was dried and weighed. Dried rhizosphere soil was used for measurement of total and available phosphorus (AP). All results were calculated on a weight basis of dried soil left in the flask after removal of the root sample.

4. Counting of PS microorganisms

The experiment included a group used for investigate organic phosphorus solubilizing microorganisms (OP-PSM) where lecithin extracted from chicken yolk was used as their sole source of P, and another group used for investigating phosphate rock solubilizing microorganisms (PR-PSM) with powdered phosphate rock (PR) from Jiangxi Province of China was used as their sole source of P.

Ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$)-yeast extract-glucose (AYG) medium (Taiwo, 1997) was used as a basic medium to determine PSM in the experiment. In the first group, 1 ml lecithin containing 2876 μg total P was added into 1000 ml medium. In the second group, 1 g powdered PR containing 395.47 mg total P was added into 1000 ml medium.

Bacteria counting was determined by inoculating 1 ml 10^{-6} – 10^{-11} soil suspension on the plates with medium. Fungi counting was done using medium plates containing filter-sterilized streptomycin (0.03%) and rose bengal (0.007%) in 10^{-5} soil suspension inoculation. Actinomycetes' counting was done by using medium plates containing $\text{K}_2\text{Cr}_2\text{O}_7$ (3.0%) in 10^{-5} soil suspension inoculation. All plates were incubated at 27 ± 2 °C. Counting of bacteria, fungi and actinomycetes was done after 1 week; PS plates were checked and counted after 2 weeks. PS ability was evaluated depending on the numbers of the developing colony within a clear zone on the agar around the colony. Final counts were expressed on a basis of one-gram-soil.

5. Measurement of rate of phosphate solubilization

Similarly, two groups of samples were formed for measuring the ability of rhizosphere PSM to solubilize OP and PR. Agar-free AYG medium was used. After OP and PR dissolved, the pH of the medium was adjusted to 6.8 and solution was then dispensed at 30 ml per flask into a series of 150 ml flasks. In the first group of OP, 1 ml lecithin containing 2876 μg total P was added into 30 ml medium. And in the second group, 30 mg powdered PR containing 359.47 mg total P was added into 30 ml medium.

Following autoclaving at 121 °C for 15 min., the flasks were inoculated with 1 ml 10^{-1} soil suspension and incubated for 21 days on a rotary shaker at 28 °C. Three flasks were used for each sample and three flask for the not-inoculated control. The contents of the culture flasks at the end of incubation period were centrifuged at 15 000 g for 20 min to remove biomass and unsolubilized matter. The soluble phosphates, expressed as equivalent phosphorus, were determined as a measure of the extent of solubilization of OP or PR (Taiwo, 1997).

RESULTS

1. PS microbes in rhizosphere of weeds

The results indicated that in absolute number, PS bacteria were dominant, then fungi; and that the number of PS actinomycetes was inconsiderable in most weed species under both P-sources (Table 1). Analysis of coefficient variation (CV) implied that PS actinomycetes number is the most sensitive to rhizospheric condition of various weed species while PS fungi is the most insensitive to rhizospheric environment of different weed species under both P-sources. Total rhizosphere PSM number derived from the sole PR P-source showed no significant difference from that from OP P-source. *Mollugo pentaphylla*, *Trisetum bifidum* and *Alopecurus aequalis* had the biggest PS bacteria number under PR P-source. *Erigeron annuus*, *Mollugo pentaphylla* and *Lysimachia clethroides* had the biggest PS fungi number while *Polygonum lapathifolium* had the biggest PS actinomycetes number under PR P-source. When OP was used as the sole P-source, *Mollugo pentaphylla*, *Erigeron annuus* and *Trisetum bifidum* showed the biggest PS bacteria number, *Mazus stachydifolius*, *Polygonum lapathifolium*, *Eragrostis pilosa* and *Elsholzia ciliata* showed the biggest PS fungus number while *Polygonum lapathifolium* showed the biggest PS actinomycetes number (Table 1). Generally evaluating, the rhizosphere of *Mollugo pentaphylla*, *Polygonum lapathifolium*, *Erigeron annuus*, *Trisetum bifidum* had more PSM number under both P-sources.

Table 1 Count of PS bacteria, fungi and actinomycetes in various weed rhizosphere (clones $10^5/g$ DW_{soil})

Weed species	The sole PR P-source				the sole OP P-source			
	B	F	A	Sum	B	F	A	Sum
<i>Conyza canadensis</i> (L.) Cronq	8.90	0.36	0.10	9.36	10.70	1.36	0.02	12.08
<i>Bidens tripartita</i> L.	6.92	1.92	0.31	9.15	8.81	4.40	0.13	13.34
<i>Lysimachia clethroides</i> Duby.	40.00	16.67	0.33	57.0	33.33	6.67	0.11	40.11
<i>Hypericum japonicum</i> Thunb.	5.89	2.21	0.04	8.14	9.20	2.94	0.07	12.21
<i>Kummerowia striata</i> (Thunb.) Schinl.	3.80	0.76	0.02	4.58	15.25	3.81	0.08	19.14
<i>Digitaria ciliaris</i> (Retz.) Koel.	2.21	0.95	0.03	3.19	3.89	3.89	0.03	7.81
<i>Viola verecunda</i> A. Gray	15.07	5.48	1.03	21.58	12.33	2.67	0.14	15.14
<i>Euphorbia humifusa</i> Willd	4.63	2.78	0.09	7.50	6.48	2.78	0.05	9.31
<i>Datura innoxia</i> Mil.	4.16	1.73	0.09	5.98	3.81	2.42	0.07	6.30
<i>Elsholzia ciliata</i> (Thunb.) Hyland	20.31	12.90	0.04	33.25	28.28	10.80	0.07	39.15
<i>Mazus stachydifolius</i> (Turcz)	13.24	8.90	0.03	22.17	76.62	16.81	0.09	93.52
<i>Mollugo pentaphylla</i> L.	244.96	17.00	0.40	262.36	245.8	8.50	0.04	254.34
<i>Eragrostis pilosa</i> (L.) Beauv.	3.89	0.59	0.02	4.50	12.87	10.90	0.02	23.79
<i>Polygonum lapathifolium</i> L.	38.40	9.50	13.00	60.90	151.0	11.50	1.50	164.00
<i>Gnaphalium affine</i> D. Don	24.30	4.80	1.70	30.80	80.90	1.00	0.03	81.93
<i>Mazus japonicus</i> (Thunb.) O. Kuntze	30.00	1.50	2.50	34.00	135.40	1.00	0.01	136.41
<i>Alopecurus aequalis</i> Sobol.	70.00	2.00	3.00	75.00	115.70	1.00	0.02	116.72
<i>Trisetum bifidum</i> (Thunb.)	80.00	7.50	1.00	88.50	150.00	4.00	0.01	154.01
<i>Erigeron annuus</i> (L.) Pers.	28.00	19.00	3.30	50.30	152.00	2.80	0.55	155.35
Average	33.931	6.1342	1.4222	41.487	65.914	5.2237	0.1585	71.296
Standard variation	55.693	6.1718	3.0005	59.493	71.964	4.4448	0.3461	72.842
Coefficient of variation	164.14	100.61	210.98	143.31	109.18	85.09	218.33	102.17

Notes: B, Bacteria; F, Fungi; A, Actinomycetes.

2. Rate of phosphate solubilizing in weed rhizosphere

Available phosphorus released by rhizosphere PSM under both sole PR P-source or OP P-source into media is shown in Table 2. AP released to the media due to the solubilization of insoluble P by weed rhizosphere PSM reached to 2260.95 μg under the sole PR P-source and 344.20 μg under sole OP P-source. Extremely great variation of solubilizing ability of rhizosphere was found among weed species especially under OP P-source (see CV data in Table 2). PSM derived from *Mazus stachydifolius*, *Erigeron annuus* showed the strongest PSA of 6340.75 μg and 6028.35 μg under PR culture. Under OP culture, PSM derived from the rhizosphere of *Eragrostis pilosa*, *Mollugo pentaphylla* and *Mazus stachydifolius* showed stronger PSA

1301.84 μg , 1122.05 μg and 1013.21 μg than other species. A significant correlation between the PS fungi number and PS ability of rhizosphere was found ($r = 0.75$ under sole PR P-source, $P < 0.05$; $r = 0.87$ under the sole OP P-source, $P < 0.05$). No consistent relationship was found in bacteria and actinomycetes. The results implied that PS fungi play a more important role in weed rhizosphere P solubilization than bacteria and actinomycetes do.

3. Total microbe number in rhizosphere of different weed species

Total number of microbial community in rhizosphere of different weed species is listed in Table 3. The same tendency in that bacteria community is larger than the fungus and actino-

Table 2 Phosphate solubilization by rhizosphere PS microbes among weed species

Weed species	AP in media (μg) under PR P-source	AP in media (μg) under OP P-source
<i>Conyza canadensis</i> (L.) Cronq	580.11	124.9
<i>Bidens tripartita</i> L.	964.86	130.82
<i>Lysimachia clethroides</i> Duby.	1177.79	160.42
<i>Hypericum japonicum</i> Thunb.	1195.74	76.36
<i>Kummerowia striata</i> (Thunb.) Schinl.	1006.31	160.42
<i>Digitaria ciliaris</i> (Retz.) Koel.	568.27	76.32
<i>Viola verecunda</i> A. Gray	2628.26	62.15
<i>Euphorbia humifusa</i> Willd	716.26	150.65
<i>Datura innoxia</i> Mil.	627.47	103.1
<i>Elsholzia ciliata</i> (Thunb.) Hyland	4854.12	763.72
<i>Mazus stachydifolius</i> (Turcz)	6340.75	1013.21
<i>Mollugo pentaphylla</i> L.	4432.87	1122.05
<i>Eragrostis pilosa</i> (L.) Beauv.	707.46	1301.84
<i>Polygonum lapathifolium</i> L.	3135.72	847.51
<i>Gnaphalium affine</i> D. Don	2578.13	107.22
<i>Mazus japonicus</i> (Thunb.) O. Kuntze	1359.45	85.78
<i>Alopecurus aequalis</i> Sobol.	1100.89	91.36
<i>Trisetum bifidum</i> (Thunb.)	2955.32	81.89
<i>Erigeron annuus</i> (L.) Pers.	6028.35	80.11
Average \pm SV	2260.95 \pm 1895.04	344.20 \pm 421.92
CV(%)	83.816	122.580

mycetes community under both P-sources was found. The total microbial community of fungi and actinomycetes cultured under OP P-source was much larger than under PR P-source while the bacteria community was approximately same under two P-sources. It implied that the development of the fungi and actinomycetes community is more sensitive to P-source.

Coefficient analysis of the AP due to solubilization of PSM from insoluble P-sources and the total microbial community showed that the total microbial community of fungus is significantly related to AP under PR P-source. Some close relationships were also found between total community of bacteria and AP under PR P-source, and the total microbial community of actinomycetes and AP under OP P-source. But no significant correlation between the total microbial community of actinomycetes and AP under PR P-source, bacteria community and AP, fungi community and AP under OP P-source condition.

With a larger PSM community, weed enhances the labile P pool and probably indirectly enhances the microbial community in the weed rhizosphere. The present study indicated that the enhancement depends on microorganisms, P-source and the rhizosphere characteristics of weed.

DISCUSSION

PSM is an ecologically special functioning group of soil microorganisms which play an important role in the turnover of organic P and insoluble inorganic phosphate, and in the cycling of P in soil (Stevenson, 1986; Smith and Paul, 1991). The composition and dynamics of this functional group was influenced greatly by vegetation type, soil texture, soil chemical elements, and pH in soil solution (Curl, 1986; Kucey et al., 1983; Lin, 1996; Yi, 1988). It was reported that about 20% of microorganisms in soil can solubilize insoluble inorganic phosphate; and that PSA

of PSM is related to the environmental condition such as farming practices. There are many reports on the community dynamics, community components and influencing fac-

tors of PSM which use PR as P-source (Illmer and Schinner, 1992; Illmer and Schinner, 1994; Kucey et al., 1989).

Table 3 Count of total numbers of microbes in weed rhizosphere (clones $10^5/\text{g DW}_{\text{soil}}$)

Weed species	Total microbes with PR				Total microbes with OP			
	B	F	A	Sum	B	F	A	Sum
<i>Conyza canadensis</i> (L.) Cronq	150.08	2.50	3.00	155.59	245.67	22.03	151.11	418.81
<i>Bidens tripartita</i> L.	349.49	3.00	2.00	354.49	610.00	30.04	174.60	814.64
<i>Lysimachia clethroides</i> Duby.	689.66	30.00	3.00	722.63	1111.10	54.96	121.27	1287.3
<i>Hypericum japonicum</i> Thunb.	250.64	10.00	1.00	261.64	268.55	34.42	41.41	344.37
<i>Kummerowia striata</i> (Thunb.) Schinl.	222.22	5.00	1.00	228.22	47.12	40.03	50.13	137.27
<i>Digitaria ciliaris</i> (Retz.) Koel.	130.00	3.00	1.00	134.00	152.00	15.00	250.97	417.97
<i>Viola verecunda</i> A. Gray	281.16	12.00	3.00	296.16	526.34	32.33	58.43	617.09
<i>Euphorbia humifusa</i> Willd	130.06	9.00	1.00	140.06	138.94	18.65	241.74	399.33
<i>Datura innoxia</i> Mil.	120.93	13.00	1.00	134.93	293.28	15.74	35.07	344.10
<i>Elsholtzia ciliata</i> (Thunb.) Hyland	280.14	25.00	1.00	306.14	358.63	52.37	147.95	558.95
<i>Mazus stachydifolius</i> (Turcz)	310.07	35.01	1.00	346.08	116.00	159.53	197.76	473.29
<i>Mollugo pentaphylla</i> L.	835.19	35.00	4.00	874.19	993.79	1156.70	242.86	2393.4
<i>Eragrostis pilosa</i> (L.) Beauv.	115.09	3.00	1.00	119.09	70.30	23.83	726.67	820.80
<i>Polygonum lapathifolium</i> L.	574.85	21.51	95.03	691.39	572.91	302.00	118.80	993.71
<i>Gnaphalium affine</i> D. Don	524.84	11.00	5.50	541.34	190.36	566.13	11.67	768.16
<i>Mazus japonicus</i> (Thunb.) O. Kuntze	179.96	4.50	8.00	192.46	175.80	406.24	69.93	651.97
<i>Alopecurus aequalis</i> Sobol.	319.93	5.00	11.00	335.93	499.00	462.80	156.25	1118.10
<i>Trisetum bifidum</i> (Thunb.)	1149.40	22.00	6.00	1177.40	312.72	375.00	360.36	1048.1
<i>Erigeron annuus</i> (L.) Pers.	888.89	36.00	16.70	941.59	15.09	222.58	14.26	251.93
Average	394.87	15.03	8.70	418.60	352.51	210.02	166.91	729.43
SV	301.36	12.13	21.32	315.58	303.95	290.48	165.10	511.56
CV	76.319	80.69	245.16	75.389	86.224	138.31	98.92	70.132

In the agroecosystem, the main process of bio-cycling of P can be described by as follows. First of all, inorganic insoluble P is solubilized by rhizosphere PR-PSM into AP form and absorbed by the weed root system in biomass form. Weed plant and crop residue buried by farmers as manure near the root system of fruit trees is decomposed and mineralized by OP-PSM and supplied to the cultivated plants in AP form. In the cycling, PR-PSM and OP-PSM are very important to the solubilization. OP existing in the main forms of phospholipid, nucleic acid and phospholipids, usually accounts for 40% to 80% of total P in soil. Only after mineralizing or solubilizing can the OP be well absorbed by plants because plants can mainly uptake PO_4^{3-} in soil solution. It was reported that weeds have stronger ability to utilize insoluble P than most crops do. For exam-

ple, under the same conditions, the ability of *Digitaria ciliaris* to absorb P is 10.3 times that of rice, 7.5 times that of maize, and 4.7 times that of wheat do in same condition (Wang et al., 1999). Therefore weed could be considered as a very important intermediate pool in the cycling of P. Additionally, the biological fixation of P by weed-PSM complex is very helpful in preventing P loss due to runoff. However little information is available on the mineralization process of OP by PSM in soil, especially in subtropical red soil zone.

Our study showed that in similar environment or even the same plant family, the PSM number and percentage of PSM in the total soil microbial community varied greatly from 0.9% to 68.29% among various weed rhizosphere. Different weed species rhizospheres have varying

PSM communities, and have significant PSA effect on PR and OP. *Mollugo pentaphylla*, *Trisetum bifidum*, and *Alopecurus aequalis* seemingly had the biggest PR-PS bacteria number. *Erigeron annuus*, *Mollugo pentaphylla* and *Lysimachia clethroides* had the biggest PR-PS fungi number while *Polygonum lapathifolium* had the biggest PR-PS actinomycetes number. *Mollugo pentaphylla*, *Erigeron annuus* and *Trisetum bifidum* had the biggest OP-PS bacteria number; *Mazus stachydifolius*, *Polygonum lapathifolium*, *Eragrostis pilosa* and *Elsholzia ciliata* had the biggest OP-PS fungus number while *Polygonum lapathifolium* had the biggest OP-PS actinomycetes number. Generally evaluating, the rhizosphere of *Mollugo pentaphylla*, *Polygonum lapathifolium*, *Erigeron annuus*, *Trisetum bifidum* had relatively greater PSM number. It indicated that various weed rhizospheres play an important role in mineralization of PR, even OP. And the PSM in weed rhizosphere is the key to solubilization and mineralization of both PR and OP. The weed-PSM complex is very important to the cycling of P and the prevention of P loss through surface runoff in the ecosystem.

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