Application of VA Mycorrhizae and phosphate solubilizers as biofertilizers in Korea

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1. Introduction

It is well known that a considerable number of bacterial species are able to exert a beneficial effect upon plant growth. Mostly they are associated with the plant rhizosphere, so they are called as rhizobcateria. This group of bacterial has been termed plant growth promoting rhizobacteria, and among them are strains from genera such as *Alcaligenes, Acinetobacter Arthrobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium*, and *Serratia*. They are used as biofertilizers or control agents for agriculture improvement, and there are numerous researchers for the area.

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. Among the bacterial genera with this ability are *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Flavobacterium* and *Erwinia*.

Visual detection and even semi quantitative estimation of the phosphate solubilization ability of microorganisms have been possible using plate screening methods, which show clearing zones around the microbial colonies in media containing insoluble mineral phosphates (mostly tricalcium phosphate or hydroxyapatite) as the single P source.

The use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. Especially, strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers.

The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorous in soil. In addition, no significant amounts of organic acid production could be detected from a phosphate solubilizer fungus, *Penicillium* sp. It is suggested that the release of H^+ to the outer surface in exchange for cation uptake or with the help of H^+ translocation ATPase could constitute alternative ways for solubilization of mineral phosphates.

Table 1. P accumulation in cultures of different bacterial species grown on insoluble mineral phosphate substrates (mg l^{-1})

Bacterial strain	Substrate		
	$Ca_3(PO_4)_2$	Hydroxyapatite	Rock phosphate
Pseudomonas striata	156	143	22
Burkholderia cepacia	35	nd	nd
Rhizobium leguminosarum	nd	356	nd
Rhizobium leguminosarum	nd	165	nd
Rhizobium loti	nd	27	nd
Bacillus polymyxa	116	87	17
Bacillus megaterium	82	31	16
Citrobacter freundi	16	7	5

nd indicates not determined

An alternative approach for the use of phosphate-solubilizing bacteria as microbial inoculants is the use of mixed cultures or co-inoculation with other microorganisms. This evidence points to the advantage of the mixed inoculations of PGPR strains comprising phosphate-solubilizing bacteria.

On the other hand, it has been postulated that some phosphate-solubilizing bacteria behave as mycorrhizal helper bacteria. It is likely that the phosphate solubilized by the bacteria could be more efficiently taken up by the plant through a mycorrhizal pipeline between roots and surrounding soil that allows nutrient translocation from soil to plant.

Considerable evidence supports the specific role of phosphate solubilization in the enhancement of plant growth by phosphate-solubilizing microorganisms. However, not all laboratory or field trials have offered positive results. Therefore, the efficiency of the inoculation varies with the soil type, specific cultivars, and other parameters.

2. General researches related to the biofertilizers in Korea

Bio-fertilizer is one of the materials expected in the environmental agriculture. On these reasons, bio-fertilizer is accepted as an important material sustainable agriculture in Korea. So the amount of bio-fertilizer using in agriculture is increasing little by little. Science papers related to the bio-fertilizers recorded in *Korean journal of soil science and fertilizer* are listed in table 2.

Table 2. Number of papers related to biofertilizers in KJSSF

Microbial inoculants	Number of papers in Years			Total
	'80	'81 '90	'91 '02	
Rhizobium	7	41	28	76
Mycorrhizae	-	4	10	14
Phosphate solubilizer	-	-	6	6
Plant growth regulator	-	4	11	15
Algae	-	1	2	3
Other	2	14	1	17
	10	65	58	133

Several results related to *Rhizobium*, mycorrhizas and phosphate solubilizers reported in the journal are as follows.

1) *Rhizobium*

Some physiological characteristics and nitrogen fixation activity under free-living conditions of indigenous rhizobia, and the host specificities between soybean varieties and *rhizobium japonicum* were studied in 1986. Results of the dissimilartory nitrate reduction and protein characteristics of indigenous soybean rhizobia, and the degrees of acid tolerance of rhizobia from the pastures in Yeongnam area could find in 1987's reports.

In 1988, effects of inoculation with selected *Rhizobium japonicum* on the yield, nodule formation and nitrogen fixing activity of soybean (*Glycine max*); the strain recognition and classification of Korean native *Rhizobium japonicum* by seroimmunological method; and the effects of co-inoculation of *R. japonicum* and *A. lipoferum* on the effectiveness of symbiotic nitrogen fixation with soybean were investigated.

Researches about selection of acid tolerant *R. meliloti* in vitro and inoculation effect in soils; nodulation and growth of *Trifolium subterraneum* cultivar as affected by pH, effects of *Rhizobium* inoculation and nitrogen concentration on the nodulation, total and allantoin nitrogen contents of the plants, and contents of crude fat and protein of soybean seeds in nutri-culture; and the effects of *Rhizobium* inoculation the changes of ureide-N and amide-N concentration in stem and root exudates of soybean plant were performed in 1989.

Studies under the titles of symbiotic potentials of *Bradyrhizobium japonicum* populations and their colony morphological characterrristics in Yeongnam soils; effect of fertilizer N application and *Bradyrhizobium* sp. (*Vigna*) inoculation on symbiotic N^2 fixation of peanut at newly reclaimed soil; inoculation effect of *R. meliloti* “YA03” to alfalfa on hilly acid soil; and the effect of inoculation with *Bradyrhizobium* sp. and lime types on peanut plant at newly reclaimed area were scrutinized in 1991.

Data related to the investigations of preparation of antibiotic-resistant *Bradyrhizobium japonicum* and its inoculation effects on soybean [*Glycin max* L. Merr]; *Rhizobium meliloti* populations and alfalfa yields due to nitrogen fertilization and inoculation methods at cultivated upland soil; effect of inoculation of peanut *Bradyrhizobium* sp. HCR-46 on the nitrogen fixation of cowpea group legume, and the transfer of foreign genes into the *Bradyrhizobium japonicum* and their inoculation effects on soybean plants can find in the papers of 1992.

Subjects according to influence of soil temperature on growth and nodulation competition of *Bradyrhizobium* sp. strains in the rhizosphere of peanut (’93); symbiotic dynamics of *Bradyrhizobium japonicum* YCK strains according to their competitive conditions for nodulation (’93); diversity of *Bradyrhizobium japonicum* with different colony morphology in intrinsic antibiotic resistance, serological property and protein profile (’96); symbiotic effectiveness and competitiveness of *Sinorhizobium fredii* on Korean soybeans (*Glycin max* L.) (’97); and the host affinities and serological distribution of *Bradyrhizobium japonic*um indigenous to Korean upland soils (’99) were also concerned for the rhizobial inoculants. Recently, a paper related to phylogenetic analysis of the genera *Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium* and *Sinorhizobium* on the basis of internally transcribes spacer region (’02) was reported.

2) VA Mycorrhiza

A search for presence of Vesicular Aruscular endomycorrhiza was attempted using 6-year-old *Panax ginseng* roots. From the experiment, brown chlamydospores with thick wall were found in the soil of root zone and it is classified as *Glomus* species (Identification of a VA mycorrhiza in the cultured *Panax ginseng.* 1990).

The infection rates of horticultural crops such as cucumber, hot pepper, lettuce, tomato and eggplant grown under greenhouse or open-field condition ranged from 38% to 70%. Hot pepper and eggplant grown under greenhouse condition showed the highest infection being 66.0% and 70.0%, respectively. *Glomus* sp.-type spores predominated in the slightly acid soil (pH 6.3), while *Acaulospora* sp.-type spores greatly predominated in the very strongly acid field (pH 4.9) (Spore density and root colonization of the indigenous VAMF in soil of some horticultural crops. 1991).

Spores of the family Glomaceae, Acaulosporeceae, and Gigasporaceae in the order Glomales were isolated from greenhouse soils grown horticultural crop in the southern region of Korea, included those of the following species: *Acaulospora biretculata, A. appendiculata*, *A. foveata*, *A. denticulate*, *A. elegans*, *A. rehmii*, *Gigaspora gigantea*, *G. decipiens*, *Glomus ambisporum*, *G. hoi*, *G. caledonium*, *Scutellispora aurigloba*, *S. calospora*, *S. coralloidea,* and *Sclerocystis pachycaulis* (Identification of the indigenous VAMF distributed in greenhouse soil. 1991).

Total nitrogen contents in shoots of cucumber, tomato, hot pepper, eggplant, and melon were lower in the mycorrhizal plants than non-mycorrhizal one, whereas P uptake in mycorrhizal hot pepper and tomato were highly remarkable. The K contents in the shoots of mycorrhizal cucumber and eggplant were highly enhanced. Ca and Mg in shoots of tomato and melon enhanced by the inoculation of indigenous VAMF. The contents of Fe, Zn, Mn and Cu in shoots of mycorrhizal crops were higher than non-mycorrhizal plants and vice versa in case of eggplant (Effects of the indigenous VAMF inoculation on the early growth and the subsequent growth after transplanting of greenhouse grown crops. 1992).

Total biomass of mycorrhizal strawberry plants was significantly increased. Contents of phosphorus, potassium and calcium in mycorrhizal strawberry plants at harvest time were higher than non-mycorrhizal ones. (Effect of inoculation with Vesicular-Arbuscular Mycorrhizal fungi on the early growth of strawberry plantlets. 1994)

Gigaspora margarita and *Acaulospora spinosa* were chosen for this investigation. After treatment some relevant growth responses of hot pepper were measured. Regardless of soil P levels, hot peppers treated with arbuscular mycorrhizal fungi had 5 34% more fresh weight than those untreated, but the effect of inoculation time and density was not different between two species. The results of this study showed that inoculation of AMF would be effective in promoting growth of hot pepper seedlings and increase transplant adaptation due in part to the resulted higher root development (Effects of arbuscular mycorrhiza inoculation and phosphorus application on early growth of hot pepper. 1999).

The density of AMF spores in plastic film house soils was highest in the site of watermelon, and those of cucumber, melon, and hot pepper sites were followed in order. The number of AMF was in the range of 101 207 per 100g dry soil The germination rate of *Gigaspora margarita* in the range of initial pH 5 9 of the medium was more than 56%. Hyphal growth was increased as pH of the medium increased. However the germination rate of *Acaulopora spinosa* was highest in the medium of pH 9 and hyphal growth in vitro was poor and not related to pH of the medium (Density of arbuscular mycorrhizal spore of plastic film house soil in Yeongnam area and characteristics of AMF in vitro. 1999).

There was a significant correlation between mycorrhizal colonization and P uptake by trifoliate orange seedlings at lower P applications. The effectiveness of mycorrhiza on P uptake was more significant at lower P applications. Uptake of N, K, Ca, Mg and Zn by trifoliate orange seedlings also increased as mycorrhizal colonization increased in volcanic ash soils of Cheju island (Arbuscular mycorrhizae colonization and mineral nutrient uptake of *Poncirus trifoliate* seedling in volcanic ash soil. 2000).

The benefits of VAM inoculation on fruit production was discovered at only low P level and salinity. Colonization rate and number of spores increased with decreased P level and salinity where there was high correlation (r=0.858**) between both. Also improved uptake of mineral nutrients was discovered at mycorrhizal treatments in decreased P level and salinity (Effect of VA Mycorrhizal fungi on alleviation of salt injury in hot pepper. 2000).

3) Phosphate-solubilizing microorganism

The phosphate fractions and their relationships with other soil characteristics in greenhouse soils located on the southwest region in Korea were studied to demonstrate the possibility of the application of phosphate solubilizing microorganisms. Especially, available P_2O_5 of 1,193 ㎎ $㎏^{-1}$ was far more than the aiming level. The distribution of greenhouse soils classified by their total P contents was 46.1% for the range of 1,000 2,000㎎ $㎏^{-1}$, 29.6% for 2,000 3,000㎎ $㎏^{-1}$, and 12.9% for 3,000 4,000㎎ $㎏^{-1}$. And the soils containing more than 1,000㎎ $㎏^{-1}$ available P_2O_5 occupied 63.0% of the examined soils. The main forms of inorganic phosphates in greenhouse soils were Ca-P and Fe-P. The P fractions compared to total P were significantly correlated to soil pH, while available P was not so (Distribution of phosphate fractions in greenhouse soils located on southwest region in Korea. 1995).

Phosphate-solubilizing microorganisms were isolated from agricultural area in Korea. Inorganic P solubilization was directly related to the pH drop by each microorganism. *Aspergillus niger* was found to be more active in solubilizing phosphate than *Pseudomonas putida* and *Penicillium* sp. The maximum concentration of phosphorus released from each of aluminum phosphate, hydroxyapatite and tri-calcium phosphate by *Aspergillus niger* in liquid culture was 776 ppm, 665 ppm and 593 ppm, respectively when KNO_3 was added as nitrogen source. For rock phosphate, it was 411ppm with ammonium sulfate as nitrogen source (Solubilization of insoluble phosphates by *Pseudomonas putida*, *Penicillium* sp. and *Aspergillus niger* isolated from Korean soils. 1995).

Effects of inoculation with phosphate solubilizing microorganisms, *Pseudomonas putida* and *Aspergillus niger*, were studied in both acidic red-yellow and alkaline calcareous soils cropped with pimiento. With inoculation of either *Pseudomonas putida* or *Aspergillus niger*, increase in phosphorous uptake by pimiento cultivated in calcareous soil was detected on non-fertilizer, and fertilizer plots except rice straw plot (Effects of inoculation with phosphate-solubilizing microorganisms on availability and plant uptake of phosphorus in red-yellow and calcareous soils of Korea. 1996).

The uptake amounts of phosphorus by lettuce and pimiento were increased by inoculation of *Aspergillus niger* in all experimental treatments. There was negative correlation between the soil microbial biomass P and the soil phosphorus content. However the soil available phosphorus ($Y=-0.0007x^2+0.7126x-29.46$. R=0.8283**) and the phosphorus absorption of plants ($Y=0.0049x^2-2.2352x+326.34$, R=0.6350*) were significantly correlated to soil microbial biomass C on the positive section of quadric curve (Assessment on the inoculation effects of phosphate-solubilizing microorganisms by soil microbial biomass. 1996).

Enterobacter agglomerans, phosphate solubilizing bacterium (PSB), possessing a high ability to solubilize hydroxyapatite (HA) markedly developed clear zones after inoculating for 36 hours at 30℃. High performance liquid chromatography (HPLC) of the products of this strain indicated that this strain excretes mainly oxalic acid with some other organic acids. During the incubation period of *E. agglomerans*, the pH values showed an inverse correlation (r^2 = 0.933**) with solubilization of inorganic phosphate. Acid phosphatase activity of the strain was 10 15 times greater than alkaline phosphatase activity. Alkaline phosphatase activity had almost constant near zero activity across time (Hydroxyapatite solubilization and organic acid production by *Enterobacter agglomerans*. 1997).

A phosphate solubilizing bacterium, strain 60-2G, possessing a strong ability to solubilize insoluble phosphate was isolated from the rhizosphere of grass. The analysis by HPLC revealed that the strain of *Enterobacter intermedium* 60-2G produced mainly gluconic and 2-ketogluconic acids with small amounts of lactic acid in broth culture medium containing hydroxyapatite. During the incubation period of the strain in broth culture, pH of the medium decreased to 3.8 while the soluble phosphate concentration increased. The reversed correlation between pH and soluble phosphate concentration indicated that the solubility of P was due to the produced organic acids. The sequence homology of the deduced amino acids suggested that *E. intermedium* 60-2G synthesized PQQ, which is essential for the oxidation of glucose by dehydrogense (Organic acid production and phosphate solubilization by *Enterobacter intermedium* 60-2G. 2002).

3. Microbial products (biofertilizer) in Korea

The microorganisms used for microbial products were *Bacillus, Pseudomonas, Lactobacillus*, photosynthetic bacteria, and nitrogen fixing bacteria, fungi of *Trichoderma* and yeast (table 3). Amongst the microbes, the major species was endospore-forming *Bacillus*, and the numbers of microbes used in microbial products were mainly 1 or 2 genera.

Table 3. Microorganisms used in commercial microbial products

Microorganism	Application ratio for product (%)
Bacillus spp.	38
Pseudomonas spp.	14
Streptomyces spp.	11
Lactobacillus spp.	9
Trichoderma spp.	5
Yeast	5
Photosynthetic bacteria	5
Nitrogen fixing bacteria	3
Others	10

Carriers used in solid type of microbial products are clay mineral, diatomaceous soil, white carbon as mineral, and rice bran, wheat bran, and discarded feed as organic matter (table 4). Especially, clay mineral and rice bran were used mainly. Sometimes the effects of carriers and/or supplements are understood to represent the function of microbial products. It is a key point to have deep consideration in the control of microbial products. In fact, farmers occasionally misunderstand this carrier effect as microbial action. It may be recommended that the use of gamma radiation to sterilize the microbial product used for reference plot.

Table 4. Different types of carrier using in solid form of microbial products

	Mineral					Organic matter				
	Clay mineral	Diatoma ceous soil	White carbon	Others	Total	Rice Bran	Wheat Bran	Discarded Feed	Others	Total
Number of items	16	3	2	3	24	15	5	3	4	27
(%)	66.7	12.5	8.3	12.5	100	55.5	18.5	11.1	14.8	100

The general actions of microbial products manifested by producer are plant growth stimulation, pest decrease, composting stimulation, and soil amelioration. Amongst the functions, the plant growth stimulator was main effect (table 5).

Table 5. Usage of the microbial products declared by producer

Usage	Plant growth stimulation	Pest decrease	Composting stimulation	Soil amelioration	Others
(%)	50	17	17	9	7

※ About 40% of the items are declared to have multiple effects.

4. Prospects

Phosphate-solubilizing microorganisms play an important role in plant nutrition through the increase in P uptake by the plant, and also plant growth promoting microbes are an important contributor to biofertilization of agricultural crops. Apart from fertilization, microbial P-mobilization would be the only possible way to increase available phosphate for plant. Accordingly, great attention should be paid to studies and application of new combinations of phosphate-solubilizing bacteria and other plant growth promoting rhizomicrobes for improved results.