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Effect of Hydrogel on Survial of *Serratia plymuthica* A21-4 in Soils and Plant Disease Suppression

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Survival of biocontrol agents and their effective colonization of rhizhosphere are the essential components for successful disease suppression. The effects of hydrogel supplement on bacterial survival and disease control were evaluated in pot and in the field. Addition of 2% hydrogel material to potting soil resulted in significant enhancement of colonization of biocontrol agent *Serratia plymuthica* A21-4 both in soil and rhizosphere of pepper plants. Rhizosphere colonization of *S. plymuthica* A21-4 retrieved from 40 days old pepper seedlings indicated 100 times higher bacterial population in hydrogel treated soil than in ordinary pot soil. The pepper plants sown in hydrogelated potting soil showed higher seed germination rate and the better growth of pepper plant than those in ordinary commercial pot soil. Although the suppression of *Phytophthora capsici* density in the potting soil by treatment of biocontrol agent A21-4 was not significantly different between in hydrogelated soil and ordinary potting soil, the suppression of Phytophthora blight between two treatments was significantly different. A21-4 treatment in hydrogelated potting soil was completely disease-free while same treatment in ordinary potting soil revealed 36% disease incidence. Our field study under natural disease occurrence also showed significantly less disease incidence (12.3%) in the A21-4 treatment in the hydrogelated soil compared to other treatments. Yield promotion of pepper by the A21-4 treatment in the hydrogelated potting soil was also recognized. Our results indicated that hydrogel amendment with biocontrol agent in pot soil would be a good alternative to protect pepper seedlings and increase plant yield.

Keywords **:** Biocontrol, Hydrogel, Phytophthra blight, *Serratia plymuthica*

Hydrogel is a network of polymer chains that are water-soluble, sometimes found as a colloidal gel in which water is the dispersion medium. They are natural or synthetic polymers that can contain water over 99% of their volume. Hydrogel materials are widely used in biomedical, optical purpose, sanitation, and construction industries (Yoon, 2000). Hydrogel materials are very diverse and generally have great water holding and retention capacity. Recently the hydrogel materials were also used in agricultural purposes (Du et al., 2002; Gehring and Lewis, 1980). Up to now the investigations on agricultural use of hydrogel has not been accumulated in Korea. The most of economically valuable crops in home and abroad are transplanted for the efficient management and to reduce the duration of cultivation. Therefore, plug nursery industries for rearing cash crops is become important area in agriculture. The potting soils for the growth medium is the prime factor for the rearing of seedlings in green house and water management is the most important skill to produce uniform and quality seedlings. Water absorbing hydrogel material is expected to hold available water without to expel the air in soil. Consequently it reduce excess water supply and eliminate the moisture stress.

In our previous study, a promising biocontrol agent, *Serratia plymuthica* A21-4 was isolated and the strain showed high efficacy on the control of Phytophthora blight of pepper (Shen et al., 2002a). The bacterial isolate was also proven to have plant growth promoting activity in commercial plug nurseries (Shen et al., 2002b; Shen et al., 2005). In this study, we investigated the survival of the biocontrol agents in hydrogelated potting soil and their colonization of the pepper in plant rhizosphere. The effect of hydrogel amendment on the suppression of Phytophthora blight by *S. plymuthica* A21-4 was also evaluated in pot and field condition under natural disease occurrence.

Material and Method

Microorganisms and culture condition. Strain *Paenibacillus polymyxa* H210, and *S. plymuthica* A21-4 were previously isolated and stored at -70°C with 20% glycerol (Shen et al., 2002a). The bacteria were routinely grown at 28°C in tryptic soy broth (TSB). The strains were marked with rifampicin resistance for the recovery and quantitation from

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soils. *Phytophthora capsici* Pa-61 (KACC 40476) was maintained on V8 juice agar and the inoculum for artificial disease inoculation was prepared as suspensions of zoospores collected from V8 juice agar by the previously described method (Shen et al., 2002b).

Preparation of hydrogelated potting soils. Commercial potting soil Tosilee (Sinangro Co. Jinju, Korea) was purchased and used throughout the experiments. Tosilee has midway moisture holding capacity and some nutrients for seedling growth. The hydrogel material used in the experiments was kindly manufactured and provided by Professor Ingyu Kang, Department of Polymer Science, Kyungpook National University. The hydrogel material was synthesized poly acrylonitrile grafted with starch.

Hydrogel materials were added to potting soil Tosilee to generate a potting soil with 2% hydrogel (w/w) (PS+ Hydrogel). The bacterial suspension of *S. plymuthica* A21-4 (10^8 cfu/ml) was also added to the hydrogelated Tosilee with the amount of 100 ml suspension to 100 g of potting soil to generate the (PS+Hydrogel+A21-4).

Microbial density in soil and pepper rhizosphere. The population density of the strains H210, and A21-4 in soil and pepper plant rhizosphere was determined by serial dilution plating technique on 1/10 strength of TSA containing 50 µg/ml of rifampicin. Soil samples or the roots of pepper seedlings were mixed with defined amount of water and ground with pestle and mortal and diluted with 0.1 M of $MgSO_4$ before serial dilution of the sample. The colony forming units (cfu) of *P. capsici* were also enumerated using Corn-meal agar with supplement of following antibiotics; pimaricin, 0.4 µg/ml; rifampicin 10 µg/ml; ampicillin 300 µg/ml; hymexazole 150 µg/ml; PCNB 300 µg/ml).

Seedling emergency in hydrogelated potting soil. The ordinary potting soil (PS), hydrogelated potting soil (PS+hydrogel) and A21-4 inoculated hydrogelated potting soil (PS+hydrgel+A21-4) were supplied to commercial plug nursery industries in Jinju area. More than 1,000 seeds of pepper plant variety, Nok-Kwang were sowed in each treatment. The way of watering and management for the seedling cultivation were followed by the routine cultural practices provided by the company. The rate of seedling emergency was measured 8 days after seeding up to 14 days. The seedling height, stem diameter, fresh weight of top part, and root fresh weight of pepper seedlings were measure at 40 days after seeding.

Evaluation of disease suppression in pot and in the field. The 40-day-old pepper seedlings (variety Nok-Kwang), which were grown in the PS, PS+Hydrogel and PS+ Hydrogel+A21-4, were transplanted into round pots (11 cm diameter and 15 cm deep) containing *P. capsici* inoculum (10^3 spores/g). After transplanting, typical Phytophthora blight symptom was carefully examined over time.

The disease suppression under natural production condition was also investigated in the field. The 40-day-old pepper seedlings (variety Nok-Kwang), which were grown in the PS, PS+Hydrogel and PS+Hydrogel+A21-4, were transplanted to the experimental field of Gyeongsang National University. The plot size of each treatment was 1 m x 20 m and each treatment included 3 replications. The numbers of infected plants were examined afterward and green fruits from each treatment were harvested 3 times. The method and conditions of pepper cultivation in a plastic house was followed by the routine farming practices in Jinju area.

Result

Survival of biocontrol agents in hydrogelated potting soil. Biocontrol agents, *S. plymuthica* A21-4 and *P. polymyxa* H210, were inoculated to the ordinary potting soil (PS) and the hydrogelated potting soil (PS+hydrogel). The population densities of two bacteria were measured over time. The population density of endospores-forming *P. polymyxa* H210 was not changed greatly up to 20 days after inoculation. No significant difference was showed in bacterial densities between PS and PS+hydrogel (Fig. 1). On the other hand, the survival density of *S. plymuthica* A21-4, which is a gram negative non spore- forming bacteria, was decreased dramatically in the PS, however, the population reduction of the same bacteria was much less in PS+hydrogel. There was a log unit difference between two bacterial population inoculated in PS and the PS+hydrogel potting soil at 20 days after bacterial inoculation (Fig. 1).

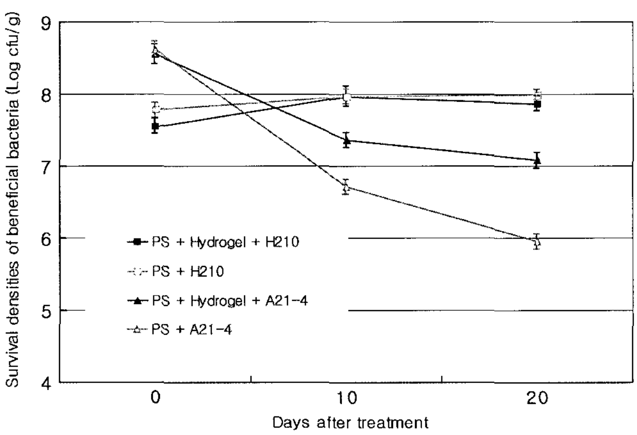

Fig. 1. Effect of hydrogel amendment in potting soil on the survival of biocontrol agents.

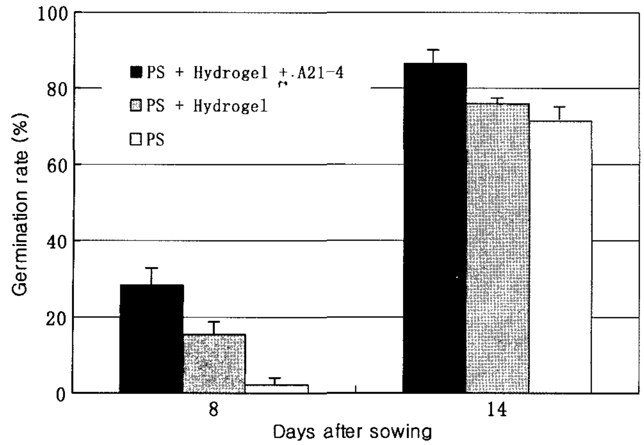

Fig. 2. Effect of hydrogel amendment in potting soil on the germination and emergency rate of pepper seedlings.

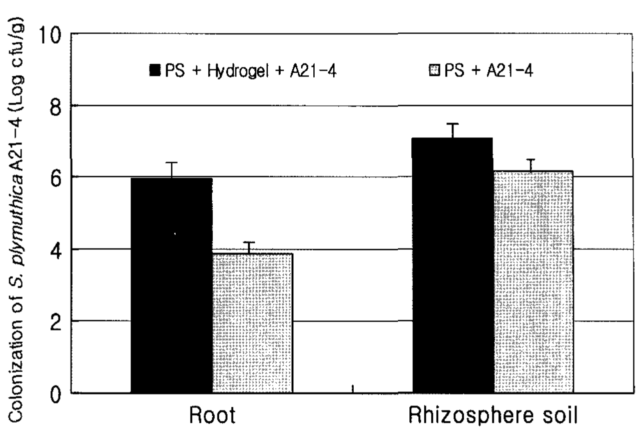

Fig. 3. Colonization of *S. plymuthica* A21-4 in the rhizosphere soil and root of pepper seedlings which were grown in hydrogelated potting soil and ordinary potting soil.

Effects of hydrogel on the growth of pepper seedlings. The effect of hydrogel on the pepper growth was investigated with pepper seedling sown in potting soils. The seed germination and emergency were more accelerated in PS+Hydrogel than in PS (Fig. 2) and the seedling growth was more uniform in the hydrogelated soil. The growth of pepper seedlings, in terms of height, stem diameter, top fresh weight, root fresh weight and chlorophyll contents, were the greater in PS+hydrogel than without hydrogel (Table 1). The additional treatment of A21-4 to PS+hydrogel brought the more pronounced growth promotion, although there was no significant difference except that shoot fresh weight was the higher by A21-4 treatment.

Colonization of *S. plymuthica* A21-4 in pepper rhizosphere. Forty days after sowing, the population densities of *S. plymuthica* A21-4 colonized in the roots of pepper seedlings and rhizosphere soils were significantly different between PS and hydrogelated potting soil PS+hydrogel. Population density of *S. plymuthica* A21-4 in the pepper root was 10^6 cfu/g in PS+hydrogel but the same bacteria in the ordinary PS was less than 10^4 cfu/g. In rhizosphere soil, A21-4 density in hydrogelated soil was over 10^7 cfu/g but the same bacterial density in ordinary PS was 10^6 cfu/g root (Fig. 3).

Effect of hydrogel on the suppression of *P. capsici* density and disease suppression by *S. plymuthica* A21-4. The treatment of A21-4 to *P. capsici* inoculated soil brought significant reduction of population density of *P. capsici*. The population density of *P. capsici* increased greatly and reached more then 1,700 cfu/g soil at 21 days after inoculation in the PS, however the fungal population in PS and PS+hydrogel treated with *S. plymuthica* A21-4 were

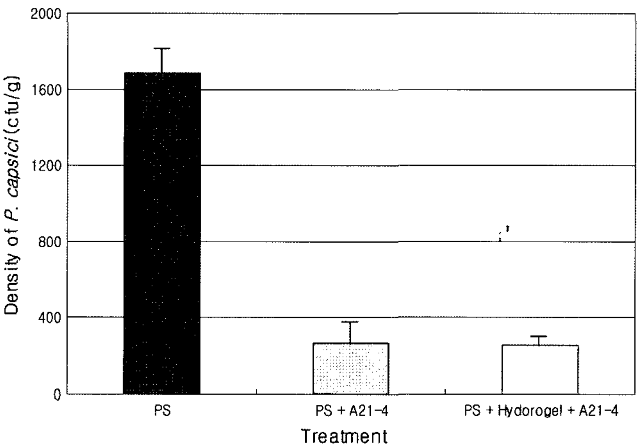

Fig. 4. Suppression of population densities of *Phytophthora capsici* by *S. plymuthica* A21-4 in hydrogel amended and ordinary potting soil.

Table 1. Enhancement of seedling growth of pepper plant by treatment of *S. plymuthica* A21-4 and hydrogel in potting soil

Treatment	Height (cm)	Stem diameter (mm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Chlorophyl (μg/mg)
PS+Hydrogel+A21-4	31.18 a[a]	5.36 a	13.29 a	1.26 a	38.75 a
PS+Hydrogel	27.99 ab	5.01 ab	10.93 b	1.38 a	37.30 a
Potting Soil (PS)	25.43 b	4.87 b	10.55 b	0.93 b	32.12 b

[a] Different letter in the column means the significant difference at 5% probability level (Turkey's studentized range test).

decreased up to 280 cfu/g and 260 cfu/g soil, respectively (Fig. 4).

Suppression of Phytophthora blight by the artificial inoculation experiments in pot was conducted in three different pot soils. The 40-day-old pepper seedlings grown in *S. plymuthica* A21-4 inoculated PS and PS+hydrogel were transplanted to round pots containing *P. capsici* inoculated potting soil. The disease occurrence was compared among treatments. The pepper seedlings without inoculation of A21-4 and hydrogel revealed 80% of disease incidence, while A21-4 inoculated PS showed 36.2% of disease incidence. Surprisingly, A21-4 inoculated seedlings grown in PS+hydrogel were completely disease-free (Table 2).

Disease suppression of the seedling reared in hydrogel with biocontrol bacteria in the field. Under natural disease occurrence in the field, the effect of hydrogel amendment and A21-4 treatment in seedling pot soil was evaluated. When the pepper seedlings grown in hydrogel amended potting soil inoculated with A21-4 or without A21-4 were transplanted in the field, the reduction of naturally occurring Phytophthora blight was significantly higher by the treatment of A21-4 in the hydrogelated potting soil. The disease incidence of pepper plants, which were grown in the ordinary potting soil, was 33.3%, however those grown in the PS+Hydrogel+A21-4 showed only 12.3% of disease incidence (Table 4). The pepper seedlings rearing in PS+hydrogel grew faster than those of in ordinary potting soil (data not shown). The fruit yield of pepper was examined 3 times after transplanting. The green fruits yield was much greater in hydrogelated potting soil than in ordinary potting soil (Table 4).

Table 2. Occurrence of Phytophthora blight in pepper seedlings which were grown in ordinary potting soil (PS), PS+*S. plymuthica* A21-4 inoculation and PS+hydrogel+A21-4

Treatment	Disease incidence (%)
PS+Hydrogel+A21-4	0.0 a[a]
PS+A21-4	36.2 b
Potting Soil (PS)	80.0 c

[a]Different letter in the column means the significant difference at 5% probability level (Turkey's studentized range test).

Table 3. Effect of *S. plymuthica* A21-4 treatment and hydrogel amendment on the green fruit yield of pepper and occurrence of Phytophthora blight in open field when the pepper seedlings grown in ordinary potting soil, PS+hydrogel, and PS+hydrogel +A21-4 were transplanted.

Treatment	Disease incidence (%)	Pepper yield[b] (kg/100 plant)
PS+Hydrogel+A21-4	12.3 a[a]	58.4 a
PS+A21-4	32.6 b	55.1 ab
Potting Soil (PS)	33.3 b	47.7 b

[a]Different letter in the column means the significant difference at 5% probability level (Turkey's studentized range test).
[b]The pepper yield was examined 3 times after transplanting.

Discussion

In recent, the use of hydrogel for agricultural purposes is become increased and various hydrogel products are available in market in USA and some European countries. The most common application of hydrogel is to mix hydrogel materials with soil to reduce the relative water loss by evaporation and drainage. However, there is no report on the application of hydrogel for plug nursery industries. We attempted to apply hydrogel to potting soil in commercial plug nurseries. Hydrogel amendment to potting soil enhanced the plant growth under same cultural practices and water management. The seed germination and emergency were accelerated and the seedling growth showed more uniformity in hydrogelated potting soil (Fig. 2). The enhanced seedling emergence and overall seedling growth might be due to greater water holding capacity of hydrogel that can reduce water loss in potting soil by drainage and evaporation.

In this experiment, *S. plymuthica* A21-4 was used to control the Phytophthra blight of pepper. Previously, we showed that *S. plymuthica* A21-4 successfully inhibited the germination of zoosporangia and cystospores, of *P. capsici* and prevented zoosporangia and zoospore formation (Shen et al., 2002b). Our previous field studies also showed that the bacteria A21-4 could successfully control the Phytophthroa blight of pepper (Shen et al., 2005). Therefore, this study was conducted to improve the biological control of Phytophthora blight of pepper plant by combining the potent biocontrol agent with hydrogel material.

Various formulations has been reported to maintain the population density of biocontrol agent in the field condition (Hur et al., 1990; Kim 1995; Nam et al., 1988; Park et al., 1989). But it is not easy to maintain sufficient population density of introduced bacteria in root or rhizosphere soil. The results obtained in this experiment revealed that amendment of hydrogel to potting soil enhanced the survival of A21-4 in soil and plant root colonization significantly (Fig. 1). Although the mechanism of hydrogel on the beneficial effect is not clear, it could be assume that hydrogel prolonged water supply to the bacteria which is sensitive to drought. The hydrogel amendment did not confer any positive effect on the survival of *P. polymyxa* H210.

Root colonization is inevitably necessary for the suppression of root pathogens (Kaiser et al., 1989; Kloepper et al., 1991; Schippers et al., 1987). Bacteria growing in or near the infection courts of roots are ideally positioned to inhibit root pathogens early in pathogenesis. It is likely that hydrogel may help *S. plymuthica* A21-4 to colonize the root system of pepper plant and to increase bacterial colonization, which may result in increased disease control efficacy.

Suppression of *P. capsici* population by A21-4 treatment in soil was not significantly different between hydrogelated and ordinary potting soil. However, suppression of Phytophthora blight of pepper in pot experiments showed great difference between two treatments. The pepper seedlings grown in PS+hydrogel+A21-4 showed no disease symptom up to 21 days after transplanting into pathogen inoculated soil. The reason for this out of accordance of two results is not clear. There might be a critical density for disease occurrence by the fungal pathogen.

Our field experiments also indicated that hydrogel amendment and A21-4 treatment for seedling rearing in potting soil has improved plant protection and plant growth promotion. Although the disease control in the open field is not perfect, the several different trials for various formulations to produce effective recipe of potting soil could be continued based on this result. The amendment of hydrogel with better biocontrol agent might enhance the biological control of Phytophthora blight of pepper.

Acknowledgments

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