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Enhancement of Biocontrol Activity of Antagonistic *Chryseobacterium* Strain KJ1R5 by Adding Carbon Sources against *Phytophthora capsici*

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Carbon utilization by *Chryseobacterium* strain KJ1R5 was studied to enhance its biocontrol activity against *Phytophthora capsici*. *Chryseobacterium* strain KJ1R5 has previously been shown to control Phytophthora blight of pepper (*Capsicum annuum* L.). Strain KJ1R5 could utilize carbon sources such as L-arabinose, D-cellobiose, β-lactose and D-galactose well. *P. capsici* could utilize D-glucose well, showing the absorbencies ranged from 0.577 to 0.767 at 600nm. When 2% L-arabinose, which could only be utilized by the biocontrol strain KJ1R5, was amended into the bacterial suspension, the efficacy of biological control increased. Among the amendments of various carbon sources into bacterial suspension, L-arabinose and D-(+)-glucose significantly enhanced biological control activity, resulting in a reduction of disease incidence to 6.9%, compared to 21.9% for the strain KJ1R5 alone and 81.3% for *P. capsici* inoculation alone, indicating that amendment with specific carbon sources could increase the biological control activity.

Keywords **:** antagonistic bacteria, biological control, *Chryseobacterium*, Phythophthora blight

Pepper (*Capsicum annuum* L.) is one of the most important crops in Korea based on consumption, nutritional value, and cash value to farmers (Hwang and Kim, 1995; Park, 1992). Pepper plants have the longest growing season of all annual crops in Korea. Pepper seeds are usually sown in February in seedling beds covered with polyethylene. Seedlings are then transplanted to fields in May. Pepper fruits are harvested from August to October, until the first frost. The Phytophthora blight of pepper caused by *Phytophthora capsici* is one of the most destructive soilborne diseases in Korea (Hwang and Kim, 1995). This blight disease results in devastating yield loss in pepper growing fields. In recent years, intensive studies of the biological control of *P. capsici* have been conducted in Korea (Jee et al., 1988; Kim and Kim, 2002; Kim et al., 2008; Sang et al., 2006, 2007).

Pepper plants can be infected by *P. capsici* in all stages of growth when environmental conditions are favorable. This disease is favored by prolonged periods of heavy rainfall accompanied by high winds from June to August in Korea. Infection of pepper plants by *P. capsici* is characterized by damping-off and sudden wilt of entire plants, which is caused by rotting of the stems near the soil surface. Stems of pepper plants are readily damaged by splitting, lodging and breaking, thus increasing the disease development. The effective control of Phytophthora blight is accomplished mainly by fungicides such as metalaxyl, oxadixyl, and propamocarb. However, due to growing environmental concerns related to ecological damage and pesticide resistance, a great deal of attention has been devoted to finding biological control agents in recent years.

Several studies have reported the protective effects of some microorganisms against soilborne pathogens (Baker et al., 1983). Antagonistic bacteria have been reported to provide biological control of Phytophthora blight of pepper (Lee et al., 1999) and antibiotic-producing bacteria antagonistic to *P. capsici* and their antibiotic activity have also been described (Kim and Hwang, 1992; Lee et al., 2004).

However, the major limitations to apply biological control agents are the inconsistent results obtained throughout the year in various field conditions. These inconsistencies may be attributed to biological activities of biocontrol agents that are not strong enough to overcome these irregularities. As a result of continuous biocontrol researches, the use of specific carbon sources as amendments to biocontrol agents was found to significantly increase the biocontrol activity against plant diseases including postharvest disease of apples by *Penicillium* species (Janisiewicz et al., 1992). It was hypothesized that a competitive advantage could be afforded to the bacterial strains introduced for biocontrol, if they were provided with a substrate that could readily be utilized as a carbon source in the bacterial formulation.

A biocontrol agent, *Chryseobacterium* strain KJ1R5 but

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not shown any antifungal activity *in vitro*, used in this study, significantly reduced Phytophthora blight caused by *P. capsici* (Kim and Kim, 2002; Kim et al., 2008). In order to amend with specific carbon sources that could be utilized only by biological control agents but not by *P. capsici*, various carbon sources were studied in conjunction with biological control agents that effectively controlled Phytophthora blight of pepper. The objectives of the present research were to find specific carbon sources that could be utilized well by the endophytic *Chryseobacterium* strain KJ1R5 only and to increase the biological control activity against *P. capsici* through the amendment with specific carbon sources.

Materials and Methods

Utilization of various carbon sources by *Chryseobacterium* strain KJ1R5. The bacterial strain KJ1R5 of *Chryseobacterium* species was streaked on tryptic soy agar (TSA) and incubated at 32°C for 3 days. After 3 days, cultures diluted in sterile distilled water were adjusted to an absorbance of 0.10 at 600 nm. Two-hundred microliters of the bacterial suspensions of strain KJ1R5 diluted in sterile distilled water were inoculated into Erlenmeyer flasks containing 100 ml Cohen-Bazire's PYGV liquid media (Cohen-Bazire G, 1957) with 2.5% of various carbon sources along with peptone, yeast extract, mineral salts and vitamins. As the basal medium, Cohen-Bazire's PYGV liquid medium was composed of 0.25 g peptone (Bacto), 0.25 g yeast extract (Bacto), 0.22 g nitrilotriacetic acid, 0.64 g $MgSO_4 \cdot 7H_2O$, 0.074 g $CaCl_2 \cdot 2H_2O$, 0.28 mg $Na_2MoO_4 \cdot 2H_2O$, 2.78 mg Na-EDTA, 12.15 mg $ZnSO_4 \cdot 7H_2O$, 7.76 mg $FeSO_4 \cdot 7H_2O$, 1.71 mg $MnSO_4 \cdot H_2O$, 0.44 mg $CuSO_4 \cdot 5H_2O$, 0.28 mg $Co(NO_3)_2 \cdot 6H_2O$, 0.19 mg $Na_2B_4O_7 \cdot 10H_2O$ per liter of distilled water, supplemented with 5 ml of filter-sterilized (0.2 μm pore size) vitamin solution containing 4 mg biotin, 4 mg folic acid, 20 mg pyridoxine-HCl, 100 mg riboflavine, 10 mg thiamine-HCl·$2H_2O$, 10 mg nicotinamide, 10 mg D-Ca-pantothenate, 0.2 mg vitamin B12, 10 mg p-aminobenzoic acid per liter of distilled water. The vitamin solution was kept at 5°C in the dark until used. The inoculated medium was shake-cultured at 32°C for 5 days, and bacterial growth was monitored every day at 600 nm using the spectrophotometer (Mecasys Co. Ltd, Seoul, Korea). The carbon sources used in this study were D-(+)-glucose, D-(+)-cellobiose, D-(+)-maltose, D-(+)-melezitose, D-mannitol, D-sorbitol, D-(+)-galactose, β-lactose, L-arabinose and glycerol (Sigma Korea, Korea).

Utilization of various carbon sources by *P. capsici*. Three mycelial plugs (5 mm in diameter) of *P. capsici* grown on V-8 juice agar, were inoculated into Erlenmeyer flasks of 100 ml of Mitchell's glucose-nitrate liquid (GNL) (Mitchell and Zentmyer, 1971a, 1971b) media containing 2.5% of various carbon sources. Mitchell's glucose-nitrate liquid medium was modified from that of Leal et al. (1967) and contained the following elements per liter: KNO_3, 0.154 g; carbon sources, 5.0 g; β-sitosterol, 0.03 g; 2-(N-morpholino)-ethanesulfonic acid (MES) as a buffer, 5.3 g; thiamine hydrochloride, 0.001 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4 \cdot 7H_2O$, 0.001 g; KH_2PO_4, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.01 g; and 1 ml of a minor element mixture that provided, in the final solution, 1 ppm of Zn ($ZnSO_4 \cdot 7H_2O$) and 0.02 ppm each of Cu ($CuSO_4 \cdot 5H_2O$), Mo ($NaMoO_4 \cdot 2H_2O$), and Mn ($MnCl_2 \cdot 4H_2O$) (Leal et al., 1967; Mitchell and Zentmyer, 1971a, 1971b; Mircetich et al., 1969). The inoculated medium was shake-cultured at 28°C for 8 days and the mycelial growth was determined from the absorbance of mycelial suspensions after mycelia were pulverized with a commercial blender at low speed; the absorbance was measured at 600 nm of using a spectrophotometer (Mecasys Co. Ltd., Korea). Carbon sources used in this study were D-(+)-glucose, D-(+)-cellobiose, D-(+)-maltose, D-(+)-melezitose, D-mannitol, D-sorbitol, D-(+)-galactose, β-lactose, L-arabinose and glycerol (Sigma Korea, Korea).

Seedling assay. Seeds were placed on two layers of moisturized filter paper (Whatman No. 2) in petri dishes and incubated at 28°C in the dark. Horticultural bed soil of TKS2 (Flora Gard Ltd., Germany) and peat moss (Acadian Peat Moss Ltd., Canada) was autoclaved twice for 40 min. The TKS2 and peat moss (1.5:1, v/v) mixtures were placed in a plastic plug tray (128 holes) (Hankuk Nongjajae, Inc., Seoul, Korea). After 3 days, seeds were sown in the plastic plug tray.

Inoculum preparation. Inoculum was prepared by the method described by Kim et al. (1997). *P. capsici* was grown on the V-8 juice agar in the dark condition at 28°C for 3 days. V-8 juice agar was composed of 200 ml V-8 juice, 17 g water agar and 2 g $CaCO_3$ per liter of distilled water. After 3 days, a mycelial disk (7mm in diameter) was cut from the margin of the growing *P. capsici*, placed in oatmeal agar and cultured at 28°C in the dark for 7 days. Oatmeal agar was composed of 30 g oatmeal and 15 g water agar per liter of distilled water. The fungal mycelia grown on oatmeal agar for 7 days were flooded with sterile distilled water and incubated under continuous fluorescent light for 5 days at 28°C for sporangial production. Five days after flooding, the mycelial culture from the oatmeal agar was chilled at 4°C for 30 min to release zoospores. the zoospores were filtrated through sterile cheesecloth and diluted with sterile water to a concentration of 1.2×10^4 zoospores per ml for the inoculation.

Effect of various concentrations of L-arabinose on biocontrol activity of *Chryseobacterium* strain KJ1R5 against *P. capsici.* A bacterial strain KJ1R5 of *Chryseobacterium* species was cultured on TSA at 32°C for 3 days. The harvested bacterial cells were suspended in L-arabinose solutions (0%, 2%, 4%, 8%), and adjusted to an absorbancy of 0.5 at 600 nm on spectrophotometer (Mecasys Co., Ltd., Korea). These solutions were then applied to saturate to the pots of pepper plants. When the pepper seedlings were 2 weeks old, one milliliter of the bacterial suspensions were applied to each pot and the pots were then inoculated with one milliliter of 1.2×10^4 zoospores per ml as previously described. Treatments groups included untreated control, pathogen only control, pathogen with KJ1R5, pathogen with KJ1R5+2% L-arabinose solution, pathogen with KJ1R5+4% L-arabinose solution, and pathogen with KJ1R5+8% L-arabinose solution. The trays were then placed in the growth chamber at 28°C/24°C (day/night, 12h/12h). Each treatment had four replicates and there were 16 plants per replication. The experiments were set up in a randomized complete block design. Disease rates were examined 14 days after inoculation.

Effect of various carbon sources on biocontrol activity of *Chryseobacterium* strain KJ1R5 against *P. capsici.* Two-week-old pepper seedlings were inoculated with the pathogen just after bacterial suspensions (1 ml) amended with or without specific carbon sources such as L-arabinose and β-lactose were applied to the pots of pepper plants. Treatment groups included untreated control, only pathogen control, pathogen with KJ1R5, pathogen with KJ1R5+2% L-arabinose solution, pathogen with KJ1R5+2% D-(+)-glucose solution, pathogen with KJ1R5+2% D-(+)-maltose solution, pathogen with KJ1R5+2% D-(+)-melezitose solution, pathogen with KJ1R5+2% D-mannitol solution, pathogen with KJ1R5+2% glycerol solution, pathogen with KJ1R5+2% D-(+)-galactose solution, pathogen with KJ1R5+2% D-sorbitol solution, pathogen with KJ1R5+2% D-(+)-cellobiose solution, and pathogen with KJ1R5+2% β-lactose solution. Then the trays were placed in the growth chamber set at 28°C/24°C (day/night, 12h/12h). Each treatment had four replicates and there were eight plants per replication. The experiments were set up in a randomized complete block design. Disease incidence was determined 14 days after inoculation by counting number of damping-off plants out of total pepper plants.

Statistical analysis. The biocontrol activity of the bacterial strain was assessed twice in a growth chamber with four replications per treatment. Results were combined and analyzed by ANOVA, and the pooled mean values were separated on the basis of least significant difference (LSD) with SigmaStat software (Systat Software, Inc., USA). Disease incidence was statistically analyzed using arcsine-transformed data.

Results

Utilization of various carbon sources by *Chryseobacterium* strain KJ1R5. The bacterial strain grew well when the concentration of carbon sources was increased to 2.5% in liquid media. Strain KJ1R5 utilized carbon sources such as L-arabinose, D-cellobiose, β-lactose and D-galactose well as shown by absorbances above 0.570 at 600 nm 5 days after inoculation. The absorbances 5 days after inoculation with D-maltose, D-glucose, glycerol, D-mannitol,

Table 1. Utilization of various carbon sources by endophytic *Chryseobacterium* strain KJ1R5

Carbon source[a]	Absorbance (at 600 nm)[b] at days after incubation				
	1 day	2 day	3 day	4 day	5 day
D-Glucose	0.138±0.005[c]	0.223±0.013	0.314±0.017	0.384±0.040	0.491±0.025
D-Cellobiose	0.180±0.037	0.227±0.075	0.457±0.095	0.554±0.089	0.665±0.020
D-Maltose	0.269±0.016	0.411±0.050	0.494±0.048	0.469±0.069	0.493±0.075
D-Melezitose	0.187±0.058	0.179±0.027	0.385±0.048	0.349±0.042	0.401±0.140
D-Mannitol	0.231±0.003	0.360±0.017	0.426±0.028	0.422±0.029	0.413±0.028
D-Sorbitol	0.177±0.007	0.167±0.004	0.182±0.006	0.157±0.005	0.156±0.001
D-Galactose	0.124±0.022	0.145±0.052	0.432±0.023	0.549±0.030	0.577±0.055
β-Lactose	0.177±0.035	0.208±0.075	0.461±0.056	0.571±0.049	0.635±0.019
L-Arabinose	0.128±0.026	0.312±0.047	0.514±0.061	0.630±0.043	0.767±0.109
Glycerol	0.213±0.019	0.336±0.038	0.384±0.044	0.374±0.038	0.438±0.023

[a]Two hundred microliters of the bacterial suspensions of strain KJ1R5 were inoculated into Erlenmeyer flasks containing 100 ml Cohen-Bazire's PYGV liquid media (Cohen-Bazire G, 1957) with 2.5% of various carbon sources with peptone, yeast extract, mineral salts and vitamins.
[b]The inoculated media was shake-cultured at 32°C for 5 days and bacterial growth was monitored at every day at 600 nm of spectrophotometer (Mecasys Co., Ltd, Korea).
[c]Mean±standard error of the replications.

Table 2. Utilization of various carbon sources by *Phytophthora capsici*

Carbon source[a]	Absorbance[b] (at 600 nm)
D-Glucose	0.427 ± 0.013[c]
D-Cellobiose	0.057 ± 0.004
D-Maltose	0.228 ± 0.018
D-Melezitose	0.046 ± 0.004
D-Mannitol	0.049 ± 0.010
D-Sorbitol	0.040 ± 0.005
D-Galactose	0.049 ± 0.002
β-Lactose	0.036 ± 0.005
L-Arabinose	0.037 ± 0.003
Glycerol	0.209 ± 0.013

[a]Three mycelial plugs (5 mm in diameter) of *P. capsici* grown on V-8 juice agar, were inoculated into Erlenmeyer flasks of 100 ml of Mitchell's glucose-nitrate liquid media containing 2.5% of various carbon sources.
[b]Mycelia were pulverized with a commercial blender at low speed for 15 sec at 8 days after incubation and the absorbances of the mycelial suspensions were determined at 600 nm.
[c]Mean±standard error of the replications.

and D-melezitose were below 0.500 at 600 nm (Table 1). However, strain KJ1R5 could not utilize D-sorbitol as shown by ane absorbance of 0.156 at 600 nm.

Utilization of various carbon sources by *P. capsici.* *P. capsici* utilized D-(+)-glucose well as a carbon source as shown by an absorbancy of 0.427 at 600 nm. D-(+)-maltose and glycerol were also utilized as carbon sources as shown above 0.200 at 600 nm (Table 2). However, *P. capsici* could not utilize L-arabinose, D-mannitol, β-lactose, D-(+)-galactose, D-(+)-melezitose, D-(+)-cellobiose, and D-sorbitol well as carbon sources as shown by the absorbances below 0.100 at 600 nm 8 days after inoculation (Table 2).

Comparison of utilization of various carbon sources by *P. capsici* and *Chryseobacterium* strain KJ1R5. Strain KJ1R5 utilized the carbon sources such as L-arabinose, D-cellobiose, β-lactose and D-galactose well as shown by absorbances above 0.570 at 600 nm. However, *P. capsici* could not utilize L-arabinose, D-cellobiose, β-lactose and D-galactose well as shown by absorbances of 0.100 at 600 nm. Among 10 carbon sources, the endophytic strain KJ1R5 highly utilized L-arabinose, while *P. capsici* did not utilize it well (Fig. 1).

Effect of various concentrations of L-arabinose on biocontrol activity of *Chryseobacterium* strain KJ1R5 against *P. capsici.* The uninoculated control showed no disease incidence; however, disease incidence of the pathogen-inoculated treatment group was significant (68.8%). Unamended strain KJ1R5 significantly reduced post emergence damping-off to 25.0% compared to the pathogen-inoculated treatment. The amendment of 2% L-arabinose into the pepper rhizosphere along with the strain KJ1R5 further significantly reduced the rate of post emergence damping-off to 10.9%, compared with that of unamended KJ1R5 as 25.0%, indicating that 2% L-arabinose enhanced the biocontrol activity of the strain KJ1R5. However, the other concentration of L-arabinose (4% and 8%) did not increase the biocontrol activity (Table 3).

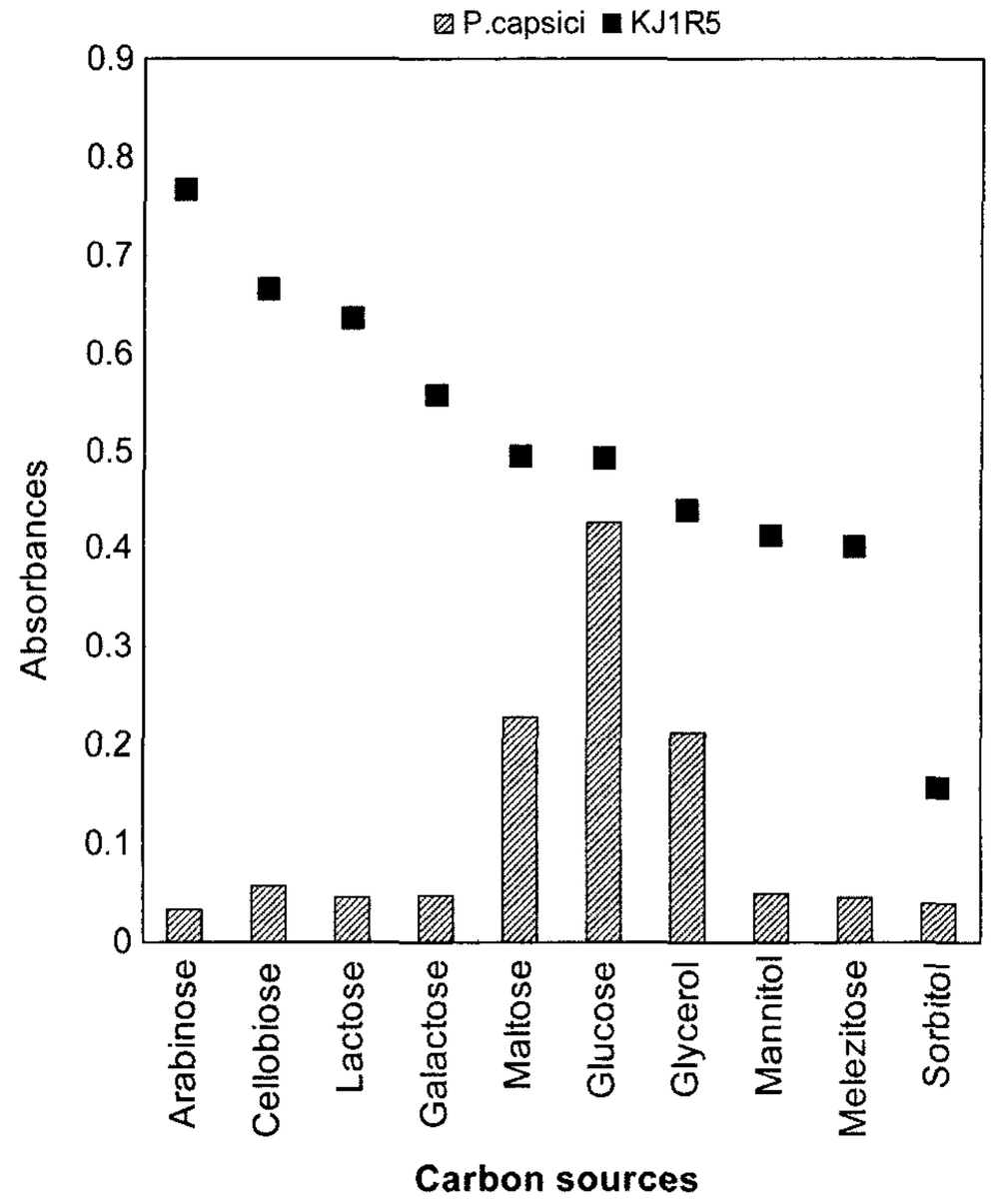

Fig. 1. Comparison on utilization of different carbon sources between *Phytophthora capsici* and antagonistic endophytic *Chryseobacterium* strain KJ1R5. Absorbances at 600 nm of *Chryseobacterium* strain KJ1R5 and *P. capsici* were determined 5 and 8 days after inoculation, respectively.

Effect of various carbon sources on biocontrol of *Chryseobacterium* strain KJ1R5 against *P. capsici.* The uninoculated control showed no disease incidence; however, disease incidence of the pathogen-inoculated treatment group was significant to be 81.3%. Strain KJ1R5 significantly reduced disease incidence to 21.9% compared to the pathogen-inoculated treatment only. Amendment of 2% L-arabinose and D-(+)-glucose into strain KJ1R5 further significantly reduced the rate of post emergence damping-off to 6.3% (Table 4). The amendment of 2% D-(+)-maltose and D-mannitol into strain KJ1R5 reduced disease incidence to 12.5% and 15.6%, respectively; however, D-sorbitol showed 75.0% disease incidence (Table 4), which was higher than that with strain KJ1R5 alone. The

Table 3. Effect of various concentrations of L-arabinose on the biocontrol activity of endophytic *Chryseobacterium* strain KJ1R5 against *P. capsici*

Treatment	Disease incidence[a] (%)
Untreated	0.0 ± 0.0 e[b]
P. capsici	68.8 ± 6.8 a
P. capsici +KJ1R5	25.0 ± 3.6 c
P. capsici + KJ1R5+ Arabinose 2%	10.9 ± 1.6 d
P. capsici + KJ1R5+ Arabinose 4%	25.0 ± 2.6 c
P. capsici + KJ1R5+ Arabinose 8%	35.9 ± 2.9 b

[a]Disease incidence was determined 14 days after inoculation. There were sixteen plants per replication and the experiment was repeated once. Statistical analysis of disease incidence was conducted with arcsine-transformed data; however, untransformed data are presented.
[b]Mean±standard error of the replications. Means followed by the same letters are not significantly different (LSD, P=0.05).

Table 4. Effect of various carbon sources on biocontrol activity of endophytic *Chryseobacterium* strain KJ1R5 against *P. capsici*

Treatment[a]	Disease incidence[b] (%)
Untreated	0.0 ± 0.0 d[c]
P. capsici	81.3 ± 3.6 a
P. capsici +KJ1R5	21.9 ± 9.4 b
P. capsici +KJ1R5+Arabinose	6.3 ± 6.3 c
P. capsici +KJ1R5+Glucose	6.3 ± 3.6 c
P. capsici +KJ1R5+Maltose	12.5 ± 5.1 bc
P. capsici +KJ1R5+Mannitol	15.6 ± 6.0 bc
P. capsici +KJ1R5+Melezitose	21.9 ± 6.0 b
P. capsici +KJ1R5+Glycerol	21.9 ± 6.0 b
P. capsici +KJ1R5+Galactose	21.9 ± 12.9 bc
P. capsici +KJ1R5+Lactose	21.9 ± 6.0 b
P. capsici +KJ1R5+Cellobiose	25.0 ± 10.2 b
P. capsici +KJ1R5+Sorbitol	75.0 ± 13.5 a

[a]Concentration of amended carbon sources was 2.5%. There were eight plants per replication and the experiment was repeated once. Disease incidence was determined 14 days after inoculation.
[b]Statistical analysis of disease incidence was conducted with arcsine-transformed data; however, untransformed data are presented. Mean ±standard error of the replications.
[c]Means followed by same letters are not significantly different (LSD, P=0.05).

amendment of 2% D-(+)-melezitose, glycerol, D-(+)-galactose and β-lactose into the strain KJ1R5 resulted in a disease incidence of 21.9%, which was similar to that of strain KJ1R5 alone (Table 4).

Discussion

The biocontrol agent *Chryseobacterium* strain KJ1R5 utilized the carbon sources L-arabinose, D-cellobiose, β-lactose and D-galactose well as shown by absorbances above 0.570 at 600 nm 5 days after inoculation. In rank, D-maltose, D-glucose, glycerol, D-mannitol, and D-melezitose were not utilized as effectively, with absorbances below 0.500 at 600 nm at 5 days after inoculation. Moreover, the biocontrol strain KJ1R5 did not utilize D-sorbitol well as shown by and absorbance of 0.156 at 600 nm. On the other hand, *P. capsici* utilized D-(+)-glucose well as shown by an absorbance of 0.427 at 600 nm. In rank, this was followed by utilization of D-(+)-maltose and glycerol as carbon sources with absorbances above 0.200 at 600 nm. However, *P. capsici* did not utilize L-arabinose, D-mannitol, β-lactose, D-(+)-galactose, D-(+)-melezitose, D-(+)-cellobiose, and D-sorbitol well as shown by absorbances below 0.100 at 600 nm 8 days after inoculation. These results were then used to increase the efficacy of biological control by amending biocontrol strain KJ1R5 with specific carbon sources that were not utilized by *P. capsici*, the target pathogen to be controlled.

When 2% L-arabinose was amended into the bacterial suspension for application to the pepper plants, it significantly increased the biocontrol activity of the antagonistic strain KJ1R5 compared to that of strain KJ1R5 alone. Among the various carbon sources, 2% L-arabinose and D-(+)-glucose significantly enhanced biocontrol activity, reducing the disease incidence to 6.9% compared to 21.9% with strain KJ1R5 alone and 81.3% with *P. capsici* inoculation alone, indicating that amendment with specific carbon sources could increase biocontrol activity.

In our previous study, we observed that nutrient sources could influence the biocontrol activity of the strain KJ1R5 for Korean ginseng root rot caused by *Phytophthora cactorum* (Sang et al., 2006). Similarly, Janisiewicz et al. (1992) observed that disease control by biocontrol agents was enhanced by amendment with nitrogen sources. They suggested that carbonaceous substrates were not limiting in the infection courts since carbon sources were abundant there. Likewise, James and Gutterson (1986) showed that production of oomycin A, an antifungal metabolite, by *Pseudomonas fluorescens* Hv37a, a biological control agent effective against Pythium damping-off of cotton, was regulated by the glucose concentration in the growth medium. Gilbert et al. (1996) suggested that disease suppression might be related to altered development of rhizosphere communities on UW85nl (*Bacillus cereus*)-treated roots. When a growing root invades the soil habitat, the microbial community around it is disturbed, resulting in a suddenly abundant nutrient supply. Gilbert et al. (1993) also reported that there were no significant bacterial community size differences between the control soybean rhizosphere and nonrhizosphere. However, bacteria from the control soybean rhizosphere utilized significantly more simple carbon sources than bacteria isolated from the nonrhizosphere soil, implying a qualitative manifestation of the rhizosphere effect. Chun (1997) reported that amendment

with specific carbon sources only utilized by the biocontrol agent *Bacillus megaterium* 91-51 could significantly increase the efficacy of biocontrol control of rice seedling disease in water-seeded rice. However, amendment with specific carbon sources did not increase the population size of the introduced bacterial biocontrol agent, suggesting that effective disease suppression might be attributed to an alteration in the qualitative difference of the rhizosphere due to amendment of specific carbon sources and the introduced biocontrol agent, rather than an increase of the population size of the biocontrol agent. Similar results were also reported in a study of biocontrol of rice seedling disease in water-seeded rice by amendment with specific carbon sources such as L-arabinose and D-Galactose into the biocontrol agent *Bacillus cereus* D324 (Sim, 2005). Each of these studies suggest that carbon and nutrient sources can affect the biocontrol activity of a biocontrol agent.

In the present study, we clearly demonstrated that the amendment with specific carbon sources into bacterial suspensions significantly increased the efficacy of biocontrol activity of *Chryseobacterium* strain KJ1R5 against *P. capsici*, suggesting that specific carbon sources could be applied to increase its biocontrol efficacy. In future studies, population analyses should be conducted in relation to the amendment with specific carbon sources.

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References

Ahmed, A. S., Consuelo, P. S. and Maria, E. C. 2000. Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. *Eur. J. Plant Pathol.* 106:817-824.

Baker, R., Stavely, C. A., Thomas, M. Sasse., and MacFall, J. S. 1983. Inhibitory effect of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust pustules on bean leaves. *Phytopathology* 73:1148-1152.

Chun, S. C. 1997. Etiology and biological control of rice seedling disease in water-seeded rice. *Ph.D. Thesis.* Louisiana State University, Baton Rouge, USA.

Cohen-Bazire, G., Sistrom, W. R. and Stanier, R. Y. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. *J. Cell Physiol.* 49:25-68.

Gilbert, G. S., Parke, J. L., Clayton, M. K. and Handelsman, J. 1993. Effects of an introduced bacterium on bacterial communities on roots. *Ecology* 74:840-854.

Gilbert, G. S., Clayton, M. K., Handelsman, J. and Parke, J. L. 1996. Use of cluster and discriminant analyses to compare rhizosphere bacterial communities following biological perturbation. *Micro. Ecol.* 32:123-147.

Hwang, B. K. and Kim, C. H. 1995. Phytophthora blight of pepper and its control in Korea. *Plant Dis.* 79:221-226.

James, D. W. and Gutterson, N. I. 1986. Multiple antibiotics produced by *Pseudomonas fluorescens* Hv37a and their differential regulation by glucose. *Appl. Environ.* 52:1183-1189.

Janisiewicz, W. J., Usall, J. and Bros, B. 1992. Nutritional enhancement of biocontrol of blue mold on apples. *Phytopathology* 82:1364-1370.

Jee, H. J., Nam, C. G. and Kim, C. H. 1988. Studied on biological control of Phytophthora blight of red-pepper. I. Isolation of antagonists and evaluation of antagonistic activity *in vitro* and in greenhouse. *Korean J. Plant Pathol.* 4:305-312.

Jiang, Z. Q., Guo, J. H., Li, S. M., Guo, Y. H. and Qi, H. Y. 2005. Evaluation of biocontrol efficiency of different *Bacillus* preparation and filed application methods against Phytophthora blight of bell pepper. *Biol. Cont.* 36:216-223.

Jung, H. K. and Kim, S. D. 2004. Selection and antagonistic mechanism of *Pseudomonas fluorescens* 4059 against Phytophthora blight disease. *Kor. J. Microbiol. Biotechnol.* 32:312-316.

Kim, B. S. and Hwang, B. K. 1992. Isolation of antibiotic-producing bacteria antagonistic to *Phytophthora capsici* from pepper-growing soils and evaluation of their antibiotic activity. *Korean J. Plant Pathol.* 8:241-248.

Kim, H. S. and Kim, K. D. 2002. Field tests of antagonistic bacteria for biological control of Phytophthora blight of pepper. *Plant Pathol. J.* 18:349.

Kim, H. S., Sang, M. K., Jeun, Y.-C., Hwang, B. K. and Kim, K. D. 2008. Sequential selection and efficacy of antagonistic rhizobacteria for controlling Phytophthora blight of pepper. *Crop Prot.* 27:436-443.

Kim, K. D., Nemec, S. and Musson, G. 1997. Control of Phytophthora root and crown rot of bell pepper with composts and soil amendments in the greenhouse. *Appl. Soil Ecol.* 5:169-179.

Leal, J. A., Gallegly, M. E. and Lilly, V. G. 1967. The relation of the carbon-nitrogen ratio on the basal medium to sexual reproduction in species of *Phytophthora*. *Mycologia* 59:953-964.

Lee, J. Y., Kim, H. S., Kim, K. D. and Hwang, B. K. 2004. *In vitro* anti-oomycete activity and in vivo control efficacy of phenylacetic acid against *Phytophthora capsici*. *Plant Pathol. J.* 20: 177-183.

Lee, Y. S., Choi, J. W., Kim, S. D. and Baik, H. S. 1999. Isolation of antagonistic bacteria to *Phytophthora capsici* for biological control of Phytophthora blight of red pepper. *Kor. J. Life Sci.* 9:1-7.

Mircetich, Srecko. M. and Zentmyer, G. A. 1969. Effect of carbon and nitrogen compounds on germination of chlamydospores of *Phytophthora cinnamoni* in soil. *Phytopathology* 59:1732-1735.

Mitchell, D. J. and Zentmyer, G. A. 1971a. Effects of oxygen and carbon dioxide tensions on sporangium and oospore formation by *Phytophthora* spp. *Phytopathology* 61:807-811.

Mitchell, D. J. and Zentmyer, G. A. 1971b. Effects of oxygen and carbon dioxide tensions on growth of several species of *Phytophthora*. *Phytopathology* 61:787-791.

Park, H. G. 1992. Current status, problems and prospects of hot pepper industry in Korea. *J. Kor. Capsicum Res. Coop.* 1:1-12.

Sang, M. K., Oh, J. Y. and Kim, K. D. 2007. Root-dipping application of antagonistic rhizobacteria for the control of Phytophthora blight of pepper under field conditions. *Plant Pathol. J.* 23:109-112.

Sang, M .K. Chiang, M. H., Yi, E. S., Park, K. W. and Kim, K. D. 2006. Biocontrol of Korean ginseng root rot caused by *Phytophthora cactorum* using antagonistic bacterial strains ISE13 and KJ1R5. *Plant Pathol. J.* 22:103-106.

Sim, J. B. 2006. Enhancement of biological efficacy with carbon sources in rice seedling diseases of water-seeded rice. M.S. thesis. Konkuk University, Seoul, Korea.