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Full Length Research Paper

The relationship and combination effects on the promotion and disease control of rhizospheric actinomycetes and entophytic bacteria in tomato (*Solanum lycopersicum*)

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Plants benefit extensively from endophytic and rhizospheric microbes. They promote plant growth and confer enhanced resistance to various pathogens. However, the interactions of these beneficial microbes are not always well understood. In this work, the interactions of two rhizospheric actinomycete strains DK56 (*Streptomyces microflavus*), ME2 (*Micromonospora* sp.) and endophytic bacterium strains XG32 (*Pseudomonas fluorescens biovar*. IV) and DP1 (*Bacillus subtilis*) were investigated via plate and broth cultivation. The strains were tested singly and in combinations for promoting the growth of tomato seedlings and biological control against pepper blight disease. The results indicated that there was nutrient competition between *Micromonospora* sp. ME2 and the two endophytic bacterium strains while *S. microflavus* DK56 competed with *B. subtilis* DP1 for nutrient when they were co-cultured in the plate. The growth of endophytic bacteria was inhibited by actinomycetes in broth. The plant growth promoting effect of endophytic bacteria was not influenced by the presence of other strains. Three strains *S. microflavus* DK56, *Micromonospora* sp. ME2 and *P. fluorescens biovar*. IV XG2 showed antagonistic activity against *Phytophthora capsici* but they did not affect each other on the control of blight disease of pepper when co-inoculated with the sporangia suspension of *P. capsici* and the control effect on pepper blight disease of *Micromonospora* sp. ME2 could be enhanced by *B. subtilis* DP1.

Key words: Actinomycetes, endophytic bacteria, interaction, growth-promotion, disease control.

INTRODUCTION

Environmental concern over conventional agricultural fertilization and disease control measures has led to the increased interest in finding environmentally friendly alternatives. Some strategies, such as using rich and diverse consortium of biological agents were of concern to researchers. It was confirmed that the activities of rhizosphere micro-organisms could affect plant health and especially, the growth and development of the root.

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Many species of actinomycetes particularly those belonging to the genus, *Streptomyces* are well known as antifungal and antibacterial bio-control agents that inhibit several plant pathogenic fungi (Errakhi et al., 2007; Joo, 2005) and thus, promote plant growth. Recently, it was reported that endophytic bacteria may promote plant growth, suppress plant diseases and could also be used as bio-control agents (Bent and Chanway, 1998; Krishnamurthy and Gnanamanickam, 1997; Sturz and Matheson, 1996).

In most research to date, bio-control agents are applied singly to combat a pathogen (Nandakumar et al., 2001). This may partially account for the reported inconsistent

performance by bio-control preparations, because single bio-control agents are not likely to be active in all soil environments in which they are applied, neither will it be active against all pathogens that attack the host plant (Domench et al., 2006). Thus, more emphasis was laid on the combined use of two or more strains, which turned out to be more effective in reducing disease in plants than using either of them singly, as reported by several researchers, Bharathi et al. (2004); Nandakumar et al. (2001); Roberts et al. (2004) and Saravanakumar et al. (2009). Consequently, the application of a mixture of bio-control agents is likely to more closely mimic the natural situation and may, therefore, represent a more viable control strategy (De Boer et al., 1999). Effective suppression of plant diseases by bio-control agents are affected by biotic and abiotic conditions. Because different mechanisms of control may be dissimilarly influenced by those conditions, it is possible that if multiple mechanisms are involved, under a certain set of conditions one mechanism may compensate for the other (Guetsky et al., 2002).

Dunne et al. (1998) showed that strain mixtures enhanced the plant growth in terms of increased seedling emergence. Guetsky et al. (2002) showed that mixture of *Pichia guilermondii* and *Bacillus mycoides* resulted in additive activity compared with their separate application. The reports aforementioned on mixtures of bio-control agents showed that combining antagonists resulted in improved bio-control. There are also reports stating that combinations of biological control agents do not result in improved suppression of disease when compared to the separate inoculants (De Boer et al., 1999; Larsen et al., 2003; Thrane et al., 2000). De Boer et al. (1999) thought that specific interactions between bio-control strains influence disease suppression by combinations of these strains. Incompatibility of the co-inoculants can arise because biocontrol agents may also inhibit each other as well as, the target pathogen or pathogens (Leeman et al., 1996). This may result in inadequate colonization, limited tolerance to changes in environmental conditions and fluctuations in production of antifungal metabolites as reported by Nandakumar et al. (2001). A pre-requisite for successful and consistent biocontrol is the compatibility of co-inoculated micro-organisms and their co-establishment in the rhizosphere of plants and the lack of competition between them (Le Floch et al., 2009). Raupach and Kloepper (1998) have reviewed studies on combinations of biological control agents for plant diseases including mixtures of fungi, fungi and bacteria, and mixtures of bacteria. To the best of our knowledge, there are no reports in combining actinomycetes with endophytic bacteria for biological control. The objectives of this study were to investigate (i) the impacts of rhizospheric actinomycete strains on the endophytic bacterial strains, (ii) whether mixed inoculation influences the ability of the antagonistic strains to reduce severity of Phytophthora disease of pepper and to promote the growth of tomato seedlings.

MATERIALS AND METHODS

Micro-organisms

All strains used in this study were isolated by workers in our laboratory. *Streptomyces microflavus* DK56 was isolated from the rhizosphere of tomato (*Solanum lycopersicum*) with the capacity of pepper blight disease resistance and growth promotion by indole-acetic acid production. *Rhizospheric Micromonospora* sp. ME2 also showed antagonistic activity against *Phytophthora capsici* by polyene production. *Pseudomonas fluorescens biovar*. IV XG2 was originally isolated from the rhizosphere of tomato based on its ability to use 1-aminocyclopropane-1-carboxylate (ACC) as the only nitrogen source. This strain has been shown to promote the growth of cucumber seedlings and restrain disease caused by *P. capsici* by modulating ethylene level via the synthesis of the enzyme ACC deaminase (Yan et al., 2005). *Bacillus subtilis* DP1 was isolated from tomato (*Lycopersicon esculentum*) with the capability of *Verticillium tricorpus* inhibition. Both *Pseudomonas fluorescens biovar*. IV XG32 and *B. subtilis* DP1 were found as endophytic colonizers of axenic cucumber seedlings. They were all described as endophytic bacteria (Shen et al., 2008).

Surface sterilization of seeds

Seeds of tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*) were surface-sterilized with ethanol and sodium hypochlorite. Before sterilization, the seeds were immersed in sterile deionized water for 24 h. Then, they were immersed in 70% ethanol for 5 min followed by three washes in sterile deionized water. Subsequently, the seeds were incubated in 1% sodium hypochlorite solution for 15 min and washed five times with sterile deionized water. The seeds were placed in Petri dishes on moist sterile filter paper and overlaid with three layers of moist sterile filter paper. All seeds were then incubated in the dark at 28°C to accelerate germination.

Interaction tests of rhizospheric actinomycetes and endophytic bacteria

Co-culture of the strains on modified potato dextrose agar plate

Prior to inoculation, all strains were grown in the respective medium as previously described. Microbial cell numbers were determined by dilution series and plate counting. Cells were harvested, and cell suspensions containing 1×10^8 cfu/ml were used for inoculation. Spore suspensions of *S. microflavus* DK56 and *Micromonospora* sp. ME2 were coated separately on modified potato dextrose agar plate. Agar disks covered with XG32 or DP1 were made from a culture by using a puncher and placed respectively on the plates which had been inoculated with actinomycetes spores as mentioned above immediately or 3 days later. Summarily; the cultures were; DK56 with XG32, ME2 with XG32, DK56 with DP1 and ME2 with DP1. Single inoculation of each strain was used as control. The plates were incubated in a growth chamber at 28°C for 3 days. Meanwhile, bacterial suspension of XG32 or DP1 was coated on the PDA plate one day prior to the respective inoculation of agar disks covered with actinomycetes hyphae of *S. microflavus* DK56 or *Micromonospora* sp. ME2, using the method previously described.

Mixed cultivation of the strains in broth

Spore suspension (10^8 cfu/ml) of *S. microflavus* DK56 and

Micromonospora sp. ME2 was mixed respectively with XG32 and DP1 at the ratio of 1: 1(v/v). 400 µl of each mixture was inoculated into 30 ml modified potato dextrose broth and cultivated on a shaker at 150 rpm at 28°C. Pure culture of each strain at the same condition was used as control. CFU of endophytic bacteria were investigated by plate-counting on peptone-beef extract agar plate after inoculation 12, 24, 36, 48, 60 and 72 h, respectively. The mycelia dry weight of actinomycetes strains was tested 6 days after inoculation.

Tests for growth-promoting activity

Plates were prepared in three steps: filter was immersed in Gauze's synthetic broth and dried, and the filter placed on the plate and then sterile by autoclaving. Strains were mixed at the ratio of 1: 1 (v/v). The dual cultures were DK56-XG32, DK56-ME2, DK-DP1, XG32-DK56, XG32-DP1, and XG32-ME2. 7 ml of each mixture was inoculated by pipetting into the plate as described earlier. Twenty alexipharmic germinated seeds (method described previously) of tomato were placed on every plate. Cell suspension of XG32 and DK56 was respectively diluted by 1: 1 ratio and then inoculated into the plate as control. All plates were incubated at 28°C for 4 days, lengths of root and hypocotyledonary axis, dry weights and fresh weights of shoot were measured. There were three replicates for each treatment.

Tests for disease control effect

The combinatorial control effect on disease caused by *P. capsici* was investigated using the method as follow: Spore suspension (10^8 cfu/ml) of *S. microflavus* DK56 and *Micromonospora* sp. ME2 were mixed respectively with cell suspension (10^8 cfu/ml) of *P. fluorescens biovar*. IV XG2 at the ratio of 1:1 (v/v). 2 ml of each mixture was transferred to Gause's synthetic agar plate and 10 aseptic pepper seedlings were transplanted to each plate followed by an inoculation of 500 µl spore suspension (10^8 cfu/ml) of *P. capsici* on the same day or 3 days later. The respective inoculation of XG32, DK56 and ME2 (cell suspension was diluted by 1: 1 ratio) with *P. capsici* served as positive control. The negative control was the inoculation of 2 ml water with 500 µl of spore suspension of *P. capsici* and 2.5 ml water inoculation served as blank control. Seedlings were maintained in an illumination chamber at 28°C for 5 days and disease development in terms of disease incidence, incidence rate and control effect were estimated. The influence of *B. subtilis* DP1 on the control effect of other three strains against *P. capsici* disease was studied in the same way. Disease development on each pepper plant was evaluated by using a 0 to 5 arbitrary scale (0 = seedlings showing no symptoms; 1 = slight browning of the root, 2 = severe browning of the root, seminal leaves symptoms absent; 3 = seminal leaves browning; 4 = complete wilt). Disease index, incidence rate and control efficiency were calculated with formula as follows:

$$\text{Disease index (DI)} = \frac{\sum(\text{rating no.} \times \text{no. of plants in the rating})}{\text{Total no. of plants} \times \text{highest rating}} \times 100\%$$

$$\text{Incidence rate} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants}} \times 100\%$$

$$\text{Control efficiency (\%)} = \frac{(\text{DI of control plot} - \text{DI of treatment plot}) \times 100}{\text{DI of control plot}}$$

Statistical analysis

Data were analyzed by Student's Newman-Keuls test. Level of significance was set at $p < 0.05$. All calculations were performed with SPSS for Windows software (version 11.5, SPSS Inc., Chicago, IL, USA).

RESULTS

Relationship between rhizospheric actinomycetes and endophytic bacterial strains cultured on plate and in broth

There was no inhibitory action of the strains on each other in septal inoculation, while in simultaneous inoculation, ME showed inhibition to XG32 and DP1, while DK56 indicated the depressant effect on DP1 (Table 1). The presumed reason may be that the prior inoculation enabled the endophytic bacterium strains to grow fully, extracting more nutrients from the medium which resulted in inanition. The actinomycete strains inoculated subsequently could not grow up and produce an effect on the competitor.

Figure 1 shows the individual growth curves of XG32 in pure culture as well as, in mixed culture of two strains (XG32 with DK56; XG32 with ME2). These curves show that the presence of DK56 and ME2 can neither stimulate nor inhibit the growth of XG32. On the other hand, the growth condition of actinomycetes was not significantly affected by the endophytic micro-organisms (Table 2). The growth curves of each strain studied in pure culture was similar to that in mixed culture. After cultivation for 24 h, the density of DP1 cultured with DK56 was 1.2×10^{10} cfu/ml and it was 8.0×10^9 cfu/ml in the mixed culture with ME2. However, the density of DP1 cells had reached a level (about 5.2×10^{11} cfu/ml) in single cultivation. This indicated that there was slight inhibitory action of actinomycetes on endophytic bacterium strain DP1. Nevertheless, there was no significant disparity between the numbers of DP1 axenic cultured or mixed cultured during the period of 24 to 72 h. It is thus, clear that the growth of endophytic bacteria can be influenced by actinomycetes, but cannot be inhibited completely. The extreme number of XG32 or DP1 can reach over 10^8 cfu/ml. The presumed relationship between actinomycetes and endophytic bacteria is nutrient competition.

Growth-promoting activity of rhizospheric actinomycetes and endophytic bacteria

Tomato seedlings treated with the different bacterial suspensions showed the improvement in plant growth parameters over untreated seedlings. The increase in root length and hypocotyledonary axis length was significantly higher in combined-strain treatment or single strain treatment when compared with untreated control.

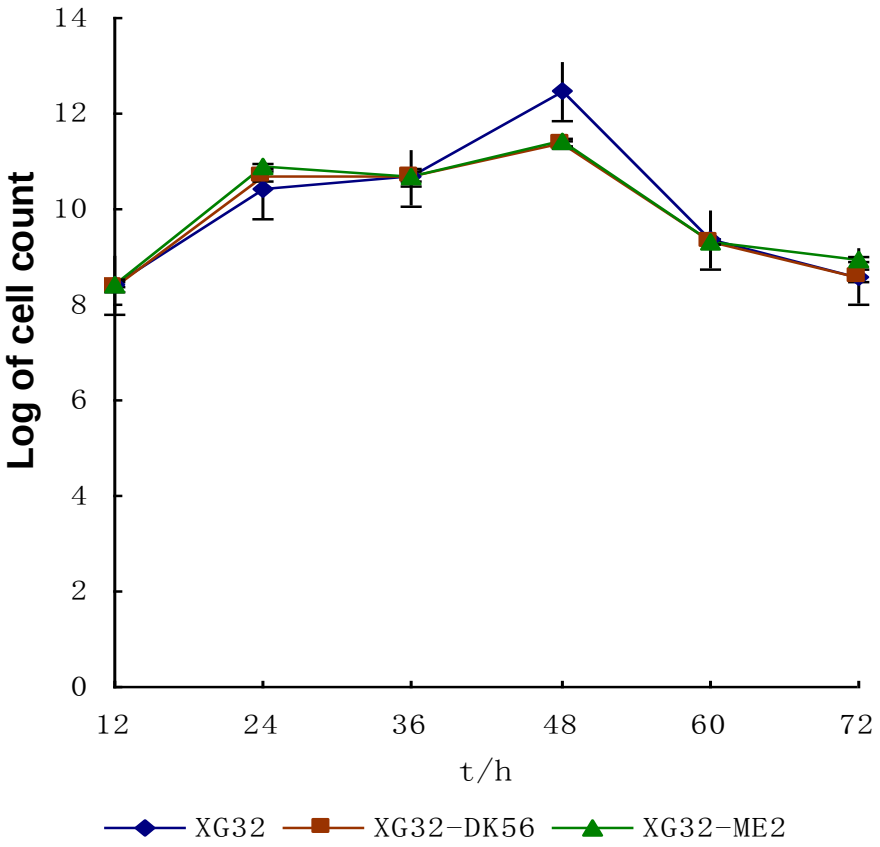

Figure 1. Growth of endophytic bacterium XG32 in mixed and individual cultures. XG32-DK56 was the mixture of strain XG32 and DK56; XG-ME2 was the mixture of strain XG32 and ME2.

Table 1. Interaction between rhizospheric actinomycetes and endophytic bacteria on modified PDA medium.

Dual cultures	Inoculation time	Inhibition (mm)	Dual cultures	Inoculation time	Inhibition (mm)
DK56-XG32	Simultaneous	–	XG32-DK56	Simultaneous	–
	At an interval of 3d	–		At an interval of 1d	–
DK56-DP1	Simultaneous	–	XG32-ME2	Simultaneous	1.1
	At an interval of 3d	–		At an interval of 1d	–
ME2-XG32	Simultaneous	–	DP1-DK56	Simultaneous	3.3
	At an interval of 3d	–		At an interval of 1d	–
ME2-DP1	Simultaneous	–	DP1-ME2	Simultaneous	1.2
	At an interval of 3d	–		At an interval of 1d	–

Inhibition (mm) = The difference of diameter of the inhibition zone and diameter of the puncher; –, no inhibition; DK56-XG32 means inoculate spore suspension of DK56 first, then inoculate the agar disks covered with XG32; XG32-DK56, Inoculate bacterial suspension of XG first and then inoculate the agar disks covered with DK56.

In addition, combined or single strain treated tomato seedlings recorded significantly higher fresh and dry weight when compared with the blank (Table 3). This indicated that the growth promotion of DK56 was not

Table 2. Mycelium dry weight of rhizospheric actinomycetes strains during mixed and individual cultures.

Treatments	Mycelium dry weight (mg)	Treatment	Mycelium dry weight (mg)
XG32-DK56	110.93 ± 8.48[a]	XG32-ME2	109.90 ± 9.14[a]
DP1-DK56	101.30 ± 2.98[a]	DP1-ME2	111.60 ± 9.33[a]
DK56	114.23 ± 4.24[a]	ME2	113.20 ± 5.98[a]

XG32-DK56, DP1-DK56, XG32-ME2 and DP1-ME2 means two strains were mixed at the ratio of 1:1 (v/v); the mycelia dry weight of actinomycetes strains was tested 6 days after inoculation. Column values followed by the same letter indicate no significant difference with p>0.05 by S-N-K test.

Table 3. The effects of actinomycetes strain DK56 in mixed and individual inoculation on the growth of tomato seedlings.

Treatments	Root length (mm)	Hypocotyl length (mm)	Fresh weight (mg)	Dry weight (mg)
DK56	48.88 ± 6.62[a]	54.71 ± 6.64[a]	366.27 ± 43.32[a]	26.17 ± 3.23[a]
DK56-DP1	50.39 ± 7.10[a]	53.04 ± 6.60[a]	407.37 ± 28.52[a]	26.83 ± 2.11[a]
DK56-XG32	48.49 ± 4.87[a]	56.09 ± 7.48[a]	357.87 ± 47.62[a]	26.80 ± 1.73[a]
DK56-ME2	45.87 ± 4.75[a]	56.46 ± 6.45[a]	405.83 ± 12.63[a]	27.03 ± 0.50[a]
CK	37.171 ± 5.03[b]	36.90 ± 5.08[b]	293.60 ± 11.33[b]	20.37 ± 2.11[b]

Data represents 20 seedlings, the measurements were made 4 days after strains inoculation. Column values followed by the same letter indicate no significant difference with p>0.05 by S-N-K test.

Table 4. The effects of endophytic bacterial strain XG32 in mixed and individual inoculation on the growth of tomato seedlings.

Treatments	Root length (mm)	Hypocotyl length (mm)	Fresh weight (mg)	Dry weight (mg)
XG32	43.01 ± 5.75[b]	47.66 ± 6.33[b]	359.27 ± 22.31[a]	24.63 ± 1.29[a]
XG32-DK56	48.49 ± 4.87[a]	56.09 ± 7.48[a]	357.87 ± 47.62[a]	26.80 ± 1.73[a]
XG32-ME2	43.26 ± 5.40[b]	49.95 ± 6.46[b]	388.27 ± 22.31[a]	25.97 ± 2.40[a]
XG32-DP1	37.76 ± 4.35[c]	49.73 ± 9.53[b]	399.63 ± 28.20[a]	23.87 ± 1.62[a]
CK	37.17 ± 5.03[c]	36.90 ± 5.08[c]	293.60 ± 11.33[b]	20.37 ± 2.11[b]

Data represents 20 seedlings; the measurements were made 4 days after strains inoculation. Column values followed by the same letter indicate no significant difference with p>0.05 by S-N-K test.

influenced by the presence of DP1, XG32 and ME2.

The growth-promotion results revealed that tomato seedlings growth (length and weight) was significantly improved by the inoculation of the strains as compared to the control (Table 4). Maximum increase in seedling growth was observed in combined inoculation of XG32 and DK56 treatment. The increase whether in root length (48.49 mm) and hypocotyledonary axis length (56.09 mm) or in dry weight (26.80 mg) was significantly higher when compared with all other treatments.

However, when the treatment was mixed and inoculated with DP1, the strain XG32 had almost no promoting effect on the root of tomato seedlings. No significant differences were observed in seedling biomass (both fresh and dry weight) among the combined inoculation treatment. The conclusion is that the promoting effect of XG32 was not influenced with the presence of DK56, ME2 and DP1.

Control effects of rhizospheric actinomycetes and endophytic bacteria on pepper blight disease

In simultaneous inoculation with *P. capsici*, treatments of single or mixed inoculation of antagonistic strains induced significant disease protection with the reduction of disease index and incidence rate compared with the control. All three individual strain treatments significantly reduced symptoms of *P. capsici* (Table 5). Mixtures consisting of two strains showed slightly greater levels of disease suppression compared with single strains. The treatment of DK56 showed the best disease suppression when compared with the strain ME2 and XG32 in the interval inoculation treatment. The greatest magnitude of

Table 5. Control efficacy of compound rhizospheric micro-organisms to phytophthora blight disease of pepper.

Treatment	Disease index		Incidence rate (%)		Control efficiency (%)	
	Simultaneous inoculation	Inoculation at an interval of 3 days	Simultaneous inoculation	Inoculation at an interval of 3 days	Simultaneous inoculation	Inoculation at an interval of 3 days
ME2	27.8^{a}	40.0^{c}	43.3^{b}	56.7^{b}	70.0	49.5
DK56	27.8^{a}	10.0^{a}	26.7^{ab}	10.0^{a}	70.0	87.4
XG32	34.4^{a}	47.8^{c}	36.7^{b}	56.7^{b}	59.1	39.6
ME2-DK56	17.8^{a}	0.0^{a}	20.0^{ab}	0.0^{a}	78.9	100.0
ME2-XG32	16.7^{a}	30.0^{bc}	16.7^{ab}	40.0^{b}	80.2	62.1
XG32-DK56	15.6^{a}	16.7^{ab}	16.7^{ab}	16.7^{a}	81.5	78.9
Negative control	84.2^{b}	79.2^{d}	100.0^{c}	100.0^{c}	—	—
Blank control	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	—	—

ME2-DK56, ME2-XG32 and XG32-DK56 were mixture of two strains at the ratio of 1:1 (v/v); The negative control was the inoculation of the mixture of water and *P. capsici* , water inoculation served as blank control; Disease index (DI) =[Σ(rating no. × no. of plants in the rating)/total no. of plants × highest rating] ×100% ; Incidence rate = (no. of diseased plants/total no. of plants) × 100%; Control efficiency (%) = [(DI of control plot –DI of treatment plot) ×100]/DI of control plot; Column values followed by the same letter indicate no significant difference with p>0.05 by S-N-K test.

control efficiency was 100% and resulting from the treatment with strains ME2-DK56 mixture. Overall, the protection activity of each strain was not influenced when they were mixed with each other. Influence of DP1 to the other three antagonistic strains on the disease control effect was investigated. Effects of individual strains and strain mixtures on the disease caused by *P. capsici* are shown in Table 6. There was a decline in disease index and incidence rate of all treatment compared with the control. Mixtures of DP1 with the other three antagonistic strains showed slightly greater levels of disease suppression compared with single strain when inoculated with *P. capsici* at the same time. This trend was striking especially, in the treatment of co-inoculation of DP1 and ME2, and the result was similar in the treatment inoculation at an interval of 3 days. These can be supposed that the disease inhibition of ME2 could be enhanced with the presence of strain DP1. No significant differences were observed in disease index and incidence rate between single strain and mixed strains treatment (DP1-XG32 and DP1-DK56), which indicated that the presence of DP1 had not influenced the control effect of strain DK56 and XG32 (Figure 2).

DISCUSSION

This investigation demonstrated the interactions between two rhizospheric actinomycetes strains DK56, ME2 and two endophytic bacterial strains XG32 and DP1. Interactions on modified potato dextrose agar medium indicated that there was nutrient competition between strain ME2 and two endophytic stains XG32 and DP1, and that was the same between strain DK56 and strain DP1. There was no influence of two actinomycete strains on the growth of strain XG32 in broth cultivation, while the inhibitory action occurred between actinomycete strains and strain DP1. The nutrient competition between the endophytic strain DP1 and two actinomycete strains was confirmed again and the promoting activity of growth promoting strains was not influenced with the presence of the antagonistic strains. Antagonistic activity of each antagonistic strain mixed inoculums did not influence each other in the simultaneous inoculation with the spore suspension of *P. capsici*, and this was similar to the report of Larsen et al. (2003). Many studies have been carried out in order to obtain an adequate and suitable biological product that could be used for agricultural and horticultural purposes, instead of the chemical ones (Domench et al., 2006). Utilization of new fungicides that contain secondary metabolites of actinomycetes to control plant disease has become increasingly important and has shown a developing trend (Joo, 2005). Numerous reports have shown that endophytic micro-organisms have the capacity to control plant pathogens (Krishnamurthy and Gnanamanickam, 1997; Sturz and Matheson, 1996), insects (Azevedo et al., 2000) and nematodes (Hallmann et al., 1997). In some cases, they are also able to accelerate seedling emergence, promote plant establishment under adverse conditions and enhance plant growth (Bent and Chanway, 1998; Chanway, 1997). Specifically, Pseudomonas and Bacillus strains have often been used as biocontrol agents. In the current study, *P. fluorescens* strains have been reported to promote the growth of a wide range of plants and showed greater antagonistic activity on various fungal pathogens and this is in agreement with the previous reports of Radjacommare et al. (2004); Saravanakumar and Samiyappan (2007) and Vivekananthan et al. (2004). Furthermore, previous studies indicated that treatments with *B. subtilis* increased yields of several crops and exhibited inhibitory effect against plant pathogens (De Freitas et al., 1997; Kokalis-Burelle et al., 2002). There are hardly any reports about the combinations of

Table 6. Control effects of the strains inoculated individually and associated with the strain DP1 on phytophthora blight disease of pepper.

Treatment	Disease index		Incidence rate (%)		Control efficiency (%)	
	Simultaneous inoculation	Inoculation at an interval of 3 days	Simultaneous inoculation	Inoculation at an interval of 3 days	Simultaneous inoculation	Inoculation at an interval of 3 days
ME2	27.8^{b}	40.0^{bc}	43.3^{c}	56.7^{b}	70.0	49.5
DK56	27.8^{b}	10.0^{a}	26.7^{bc}	10.0^{a}	70.0	87.4
XG32	34.4^{b}	47.8^{c}	36.7^{bc}	56.7^{b}	59.1	39.6
ME2-DP1	20.0^{ab}	20.0^{ab}	20.0^{b}	20.0^{a}	76.2	74.8
DK56-DP1	23.3^{ab}	6.7^{a}	23.3^{bc}	6.7^{a}	72.3	91.5
XG32-DP1	35.6^{b}	37.8^{bc}	36.7^{bc}	50.0^{b}	57.7	52.3
Negative control	84.2^{c}	79.2^{d}	100.0^{d}	100.0^{c}	—	—
Blank control	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	—	—

ME2-DP1, DK56-DP1 and XG32-DP1 were mixtures of two strains at the ratio of 1:1(v/v); The negative control was the inoculation of the mixture of water and *P. capsici* , water inoculation served as blank control; Disease index(DI) = [Σ(rating no. × no. of plants in the rating)/total no. of plants ×highest rating] ×100%; Incidence rate = (no. of diseased plants/total no. of plants) ×100%; Control efficiency (%) = [(DI of control plot –DI of treatment plot) ×100]/DI of control plot; Column values followed by the same letter indicate no significant difference with p>0.05 by S-N-K test.

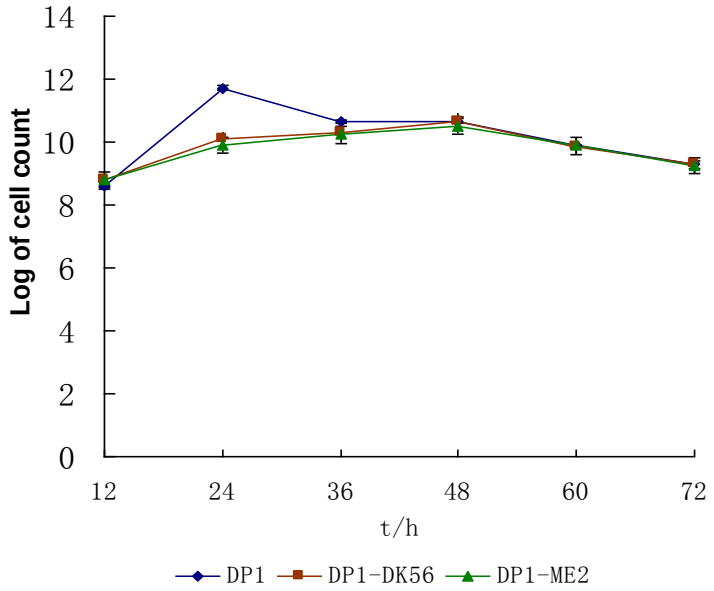

Figure 2. Growth of endophytic bacterium strain DP1 in mixed and individual cultures. DP1-DK56 was the mixture of strain DP1 and DK56; DP1-ME2 was the mixture of strain DP1 and ME2.

biological control agents of bacteria and actinomycetes for the inhibitory effect of antibiotics produced by actinomycetes on bacteria strains. In addition, there are also no reports about the combination of endophytic bacterium strains with actinomycete strains as biocontrol agents. There are many advantages of mixtures of actinomycetes and bacteria, which is in contrast with mixtures of fungi, mixtures of fungi and bacteria and mixtures of bacteria. Suppose the mixtures of endophytic bacteria and actinomycetes were applied to the soil, the endophytes would colonize the plant while actinomycete strains would remain at the rhizosphere of the plant, thus, avoiding nutrient competition and co-intervention, and thereby, greatly improving the effect of each strain. Results of promoting and antagonistic assay indicated that the four strains used in this study influenced each other slightly. In utilization, it is best to cultivate the endophytic bacteria separately until the quantity reaches a given level (generally 10^8 cfu/g) prior to the combination with the actinomycetes, thereby avoiding the decline of the control effect which may be caused by the inhibitory action from actinomycetes.

It is important to note that all experiments were carried out under the sterile condition, and when these micro-organisms are added in the field, they are bound to compete with other soil micro-organisms and survive environmental conditions that may influence their efficacy as biocontrol agents.

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