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Field Control of Phytophthora Blight of Pepper Plants with Antagonistic Rhizobacteria and DL-β-Amino-n-Butyric Acid

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Treatment with antagonistic rhizobacteria *Burkholderia cepacia* strain N9523 or an inducer of resistance DL-β-amino-*n*-butyric acid (BABA) effectively inhibited *Phytophthora capsici* infection on pepper plants in artificially infested pots. Treatment with BABA alone at 1,000 μg/ml or together with *B. cepacia* in combination induced a strong protection from the Phytophthora disease in the greenhouse. In artificially infested field, protection of pepper plants against the Phytophthora epidemic by BABA treatment was maintained at a considerable level. In contrast, soil drench with the antagonist *B. cepacia* alone, or in combination with BABA did not suppress the Phytophthora epidemic in the field. Mortality of pepper plants caused by *P. capsici* infection was significantly reduced by treatment with the antagonist *Pseudomonas aeruginosa* strain 950923-29 and BABA (12-29% plants diseased) relative to the untreated control (41-91% plants diseased) in the naturally infested field. Treatment with the antagonist *Ps. aeruginosa* strain 950923-29 and BABA in combination, the antagonist alone or BABA also resulted in high levels of protection against Phytophthora blight in pepper plants. In the plastic filmhouse test, the average percentage of plants diseased was significantly low relative to the naturally infested field. Treatment with the antagonist *Ps. aeruginosa* strain 950923-29 and BABA in combination was most effective in suppressing the Phytophthora disease in field and plastic filmhouse.

Keywords : antagonistic rhizobacteria, *Capsicum annuum*, DL-β-amino-*n*-butyric acid, *Phytophthora capsici*.

Phytophthora blight of pepper, which is caused by *Phytophthora capsici* Leonian, is one of the most devastating soilborne diseases of pepper in Korea (Kim, 1993). Intensive studies have been concentrated on the biology of *P. capsici*, evaluation of pepper germplasm for disease resistance, yield-loss assessment, and the testing of chemical, biological, and cultural measures of control (Hwang and Kim, 1995). In recent years, research on the biological control of Phytophthora blight of peppers has been expanded in response to growing concerns about the side effects of fungicides as environmental pollutants.

For the effective biological control of soilborne plant pathogens, a major consideration has been given to proliferation of the antagonist after introduction into the soil. Among the desirable attributes of a successful antagonist is its ability to produce inoculum in excess and to survive, grow, and proliferate in soil and the rhizosphere (Baker and Cook, 1974). Various actinomycetes, bacteria, and fungi, which show antagonism to *P. capsici*, exist in soils where peppers are grown (Ahn and Hwang, 1992; Jee et al., 1988; Kim and Hwang, 1992). In particular, some antagonistic rhizobacteria such as *Burkholderia cepacia* (Jee et al., 1988) and *Pseudomonas aeruginosa* (Kim and Hwang, 1992) were very effective against Phytophthora blight in pepper plants under laboratory and greenhouse conditions.

In addition to biological control, as a protection method of Phytophthora blight in pepper plants, researches for the effective control of Phytophthora blight by treatment with a nonfungicidal synthetic chemical, DL-β-amino-n-butyric acid (BABA), to pepper plants have been conducted. Induction of resistance can be attained by the abiotic inducers such as polyacrylic acid (Gianinazzi and Kassanis, 1974), acetylsalicylic acid (White, 1979), salicylic acid (White, 1979), and 2,6-dichloroisonicotinic acid (Metraux et al., 1991). Little information is available in the literature on the effects of exogenously applied amino acids on plant disease. The increase in 4-aminobutanoic acid content and glutamate decarboxylase activity have been observed in many plants under a variety of environmental stress conditions (Sayata and Nair, 1990). Papavizas (1964) showed that DL-β-amino-n-butyric acid and DL-threo-3-methylaspartic acid controlled *Aphanomyces* root rot in pea plants.

A nonprotein amino acid, aminobutyric acid, has been implicated as an inducer of resistance in a variety of pathosystems (Cohen, 1996). Of the various aminobutyric acids, DL-β-amino-n-butyric acid (BABA) effectively protected

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tomato plants against *Phytophthora infestans* (Cohen, 1993), tobacco plants against *Peronospora tabacina* (Cohen, 1994), and pepper plants against *P. capsici* (Sunwoo et al., 1996) and *Colletotrichum coccodes* (Hong et al., 1999).

In our previous study, we demonstrated that pepper plants treated with BABA were strongly protected against challenge-inoculated *P. capsici* (Sunwoo et al., 1996). More recently, further study has shown that the accumulation of pathogenesis-related proteins such as β-1,3-glucanases and chitinases and endogenous salicylic acid occurred in the induction of resistance to Phytophthora blight in pepper plants with treatment of BABA (Hwang et al., 1997).

There have been still many difficulties in biological control of Phytophthora blight of pepper plants with antagonistic rhizobacteria (Hwang and Kim, 1995). Especially, poor performance of the antagonists in field trials may be due to failure of successful colonization. The antagonist populations in field soils decreased rapidly below the threshold level of antagonistic activity within a month after application, unless they were applied frequently. Intensive researches may be required to effectively control Phytophthora blight of pepper plants with BABA, as a plant defense activator in the field or greenhouse. In the present study, protective effect of the antagonistic rhizobacteria and BABA against Phytophthora blight in pepper plants was evaluated. We further examined whether or not BABA could be available for the induction of resistance to Phytophthora blight under the greenhouse or field condition. Herewith, we also report that treatment with the antagonistic rhizobacteria and BABA in combination could cause some synergistic effects on the protection of pepper plants against Phytophthora disease.

Materials and Methods

Test of antagonist *Burkholderia cepacia* strain N9523 and BABA in potted pepper plants. Pepper (*Capsicum annuum* L.) cv. Hanbyul at first-branch stage was used in this study. Pepper seeds were sown in a plastic tray (55 cm × 35 cm × 15 cm) containing steam-sterilized soil, sand and compost (1:1:1, v/v/v). Seedlings at the two-leaf stage were transplanted to plastic pots (5 cm × 15 cm × 10 cm) containing the same soil mix. Complex fertilizer was applied to plants after transplanting. Pepper plants were raised in a growth room under 16 hr day illumination at 27±2°C.

The antagonistic rhizobacteria *Burkholderia cepacia* strain N9523 was cultured in the tryptic soy broth (TSB) in a rotary shaking incubator for 48 hr at 28°C. Cells of *B. cepacia* strain N9523 were harvested by centrifugation at 3, 500 g, washed and then resuspended in a sterile water. Bacterial suspensions were adjusted to various concentrations by a serial dilution plating method. Suspensions of *B. cepacia* strain N9523 and DL-β-amino-n-butyric acid (BABA) were drenched to pepper plants in soils before wounded stem or soil-drench inoculation with zoospore suspension of *Phytophthora capsici* (10^5 zoospores/ml).

Disease severity of Phytophthora blight in pepper plants was rated 8 days after inoculation of *P. capsici* based on a 0-5 scale : where 0 = no visible disease symptoms; 1 = leaves slightly wilted with brownish lesions beginning to appear on stems; 2 = 30-50% of entire plant diseased; 3 = 50-70% of entire plant diseased; 4 = 70-90% of entire plant diseased; 5 = plant dead. Disease severities in antagonistic rhizobacteria- or BABA-treated and untreated plants were examined.

Test of antagonist *Burkholderia cepacia* strain N9523 and BABA in artificially infested field. Pepper (*Capsicum annuum* L.) cv. Hanbyul was used in this field test. Protective effect of antagonist *Burkholderia cepacia* strain N9523 and BABA on Phytophthora blight of pepper (cv. Hanbyul) plants was evaluated in the artificially *P. capsici*-infected field in Dukso, Korea. Pepper seeds were sown on February 15, 1998 and seedling plants in pots were transplanted in field on May 2. Insecticide or herbicide was treated to pepper plants, if necessary. The antagonistic strain N9523-treated, DL-β-amino-n-butyric acid (BABA)-treated, the strain N9523 + BABA-treated, metalaxyl-treated and untreated control plots were tested in this study. Each plot was placed with 3 replicates in a randomized block design. The size of plots was 24 m^2/plot where 75 plants were grown. The 100 ml of culture of the antagonist strain N9523 (10^9 CFU/ml), BABA (1,000 μg/ml) and metalaxyl (50 g/ 20L) were soil-drenched to each of pepper plants on June 10, 30 and July 6. To inoculate *P. capsici* artificially, 10 ml of zoospore suspensions (10^5 zoospores/ml) were soil-drenched to each of pepper plants in fields on July 8. Disease incidence of Phytophthora blight in each plot was rated daily from July 14 to 28.

Formulation of antagonist *Pseudomonas aeruginosa* strain 950923-29. The antagonistic rhizobacteria *Pseudomonas aeruginosa* strain 950923-29 was cultured in the nutrient broth in a fermentor for 24 hr at 30°C. To prepare the antagonist agent, one liter of vermiculite, 10 ml-cultures of antagonistic rhizobacteria and 1% of wheat bran were mixed with sterile water so as to give 60% water content. After incubation for 20 days at 30°C, the soils mixed with antagonist agent at the rate of 9:1 was used for the cultivation of pepper plants.

Granules of antagonistic bacterial agents were prepared using cultures of *Ps. aeruginosa* strain 950923-29. Ten grams of sodium alginate was mixed with 1 L distilled water on hot plates to give final concentration of 1% (w/v). Twenty milliliters of bacterial suspension and 200 g kaolin were added to the sodium alginate solution, followed by stirring for 1 min. The mixtures in a plastic jar were slurried with 0.25 M $CaCl_2$ through the silicon tubes (ϕ 2 mm) to prepare granules. Two or three grams of the antagonist granules were applied to pepper plant at the transplanting.

Test of antagonistic bacterial agent in the naturally infested field. Field and plastic filmhouse tests of antagonistic bacterial agents were conducted in pepper field in Suwon, Korea. Cultivar Dajoa was used in this study. Antagonist *Ps. aeruginosa* strain 950923-29-treated, metalaxyl-treated, DL-β-amino-n-butyric acid (BABA)-treated, the bacterial strain 950923-29 plus BABA-treated and untreated field plots were placed with 3 replicates in a randomized block design. In case of the plastic filmhouse test, each plot was placed with 2 replicates. The size of plots was 35

m^2/plot where 68 plants were grown. Pepper seedling plants were transplanted in field on May 7. Treatment with the antagonists was done using soil mixtures, antagonist granules, and direct soil-drench. Pepper seedlings grown in the soil mixtures containing antagonistic bacterial agent were transplanted in the field and 2 3g granules of bacterial agent were applied to plant after transplanting. During the vegetation season of pepper plants, antagonistic bacterial suspensions (10^8 cfu/ml/plant) were soil-drenched 3 times every month from the beginning on June 8. Metalaxyl-copper was also foliar-sprayed 3 times every month from the beginning on June 8. BABA at 1,000 μg/ml was applied on pepper plants 5 times on May 7, 12, June 2, July 2 and August 2. Artificial inoculation with *P. capsici* was not performed in pepper-growing fields. In the plastic filmhouse test, all the treatments were done, as in the field test. Incidence of the plants diseased was rated on August 14.

Results

Protective effect of the antagonist *B. cepacia* strain N9523 and BABA on Phytophthora blight in pepper plants in artificially infested pots and fields. Treatment with antagonistic rhizobacteria *B. cepacia* strain N9523 or an inducer of resistance BABA effectively inhibited *P. capsici* infection on pepper plants at the first-branch stage. The Phytophthora blight of pepper plants was significantly reduced by the treatment with *B. cepacia* strain N9523 (10^8 cfu/ml) 5 days before inoculation of *P. capsici.* In BABA-treated plants, disease development declined remarkably at 500 and 1,000 μg/ml of BABA (no data presented). Soil drench with the antagonist *B. cepacia* did not suppress the Phytophthora development in pepper plants after stem inoculation of *P. capsici* (Fig. 1). When inoculated by soil-drench with *P. capsici*, however, pepper plants were significantly protected against the *P. capsici* infection by treatment with *B. cepacia*, especially more pronouncedly at the first branch stage than at the six-leaf stage of plants. Treatment with BABA alone at 1,000 μg/ml or together with *B. cepacia* in combination induced the strong protection from the Phytophthora disease in the inoculated pepper stems, but provided complete protection in the roots inoculated by soil drench with *P. capsici*. In all the treatments, the protection effects on the Phytophthora disease were more marked in pepper plants at the first branch stage than at the six-leaf stage. However, the synergistic effect of the BABA treatment with the antagonist strain N9523 was not found in this pot test.

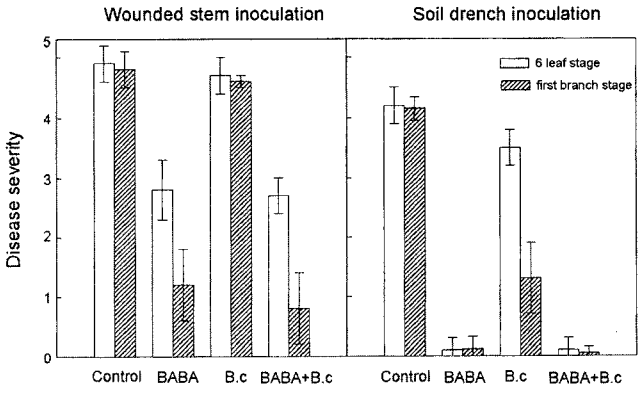

Fig. 1. Effects of *Burkholderia cepacia* strain N9523 and DL-β-amino-n-butyric acid (BABA) on protection of pepper plants at different growth stages against *Phytophthora capsici* infection using different inoculation methods. Plants at six-leaf and first-branch stages were soil-drenched with *B. cepacia* (10^9 cfu/ml) and BABA (1 mg/ml) 3 days before inoculation of *P. capsici* (10^5 zoospores/ml). Disease severity was rated 8 days after inoculation of *P. capsici* based on a 0-5 scale, where 0 = no visible symptom and 5 = plant dead. Vertical bars represent standard deviations.

The control efficacy of BABA and the antagonist *B. cepacia* strain N9523 against Phytophthora blight of pepper plants was evaluated in a pepper field artificially inoculated by *P. capsici* (Table 1). Because natural infection by *P. capsici* was not found in pepper-growing field in Dukso, zoospore suspension of *P. capsici* was inoculated by soil drench in the tested plots. In all the plots, except for metalaxyl-treated plots, the Phytophthora disease began to occur in pepper plants at 6 days (July 14) after inoculation of *P. capsici* in the fields. Protection of pepper plants against the Phytophthora epidemic by BABA treatment was maintained at a considerable level by July 20. Application of metalaxyl resulted in the complete control of the Phytophthora disease by July 20. In contrast, soil drench with the antagonist *B. cepacia* alone, or in combination with BABA did not suppress the Phytophthora epidemic, indicating that treatment with *B. cepacia* culture may not affect *P. capsici* infection in pepper-growing field.

Protective effect of the antagonist *Ps. aeruginosa* strain

Table 1. Protective effect of DL-β-amino-n-butyric acid (BABA) and antagonistic bacteria *Burkholderia cepacia* strain N9523 on Phytophthora blight in the pepper field in Dukso, Korea in 1998

Treatment[a]	% plants diseased on the date			
	7/14	7/16	7/20	7/28
BABA (A)	1.4±0.1[b]	8.8±7.8	45.3±1.7	100±0.0
B. cepacia (B)	15.9±7.2	38.5±7.3	78.9±9.4	100±0.0
(A) + (B)	3.8±3.3	19.7±16.4	55.8±20.7	98.3±2.9
Metalaxyl	0.0±0.0	0.0±0.0	0.0±0.0	19.2±5.2
Untreated	3.9±3.1	17.9±7.5	68.8±10.6	99.5±0.9

[a] The antagonistic *B. cepacia* strain N9523 (10^9 cells/ml tryptic soy broth), BABA (1,000 μg/ml) and metalaxyl-mancozeb (50 g/20 L) were soil-drenched in the pepper field on June 10, June 30 and July 6. The zoospore suspension (10^5 zoospores/ml) of *P. capsici* was inoculated by soil-drench in the field on July 8.

[b] Disease incidence was rated daily after appearance of Phytophthora blight on pepper plants. Values represent means±standard deviations.

Table 2. Effects of antagonistic *Pseudomonas aeruginosa* strain 950923-29 and BABA application on incidence of Phytophthora blight of pepper plants in fields, Suwon, Korea, in 1998

Treatment	% plants diseased			Average
	Rep I	Rep II	Rep III	
Antagonistic agent(A)	18.1	17.6	19.4	18.4±0.5
BABA(B)	29.2	20.6	26.4	25.4±2.5
(A) + (B)	13.9	11.8	9.7	11.8±1.2
Metalaxyl-copper	9.7	2.9	13.9	8.8±3.2
Untreated control	91.7	41.2	44.4	59.1±16.3

Table 3. Effect of antagonistic *Pseudomonas aeruginosa* strain 950923-29 and BABA application on incidence of Phytophthora blight of pepper plants in the plastic filmhouse, Suwon, Korea, in 1998

Treatment	% plants diseased		Average
	Rep I	Rep II	
Antagonistic agent(A)	0	12.5	6.2±6.2
BABA(B)	16.7	4.2	10.4±6.2
(A) + (B)	0	4.8	2.4±2.4
Metalaxyl-copper	4.2	12.5	8.4±4.2
Untreated control	14.3	14.3	14.3±0.0

950923-29 and BABA on Phytophthora blight of pepper plants in the naturally infested field. Mortality of pepper plants caused by *P. capsici* infection was significantly reduced by the treatment with the antagonistic bacterial agent strain 950923-29 and DL-β-amino-n-butyric acid (BABA) (12-29% plants diseased) relative to the untreated control (41-91% plants diseased) in naturally infested field (Table 2). Application of metalaxyl-copper was most effective in controlling Phytophthora blight in pepper plants (average 8.5% plants diseased). Treatment with the antagonist strain 950923-29 and BABA in combination, the antagonist alone or BABA also showed high levels of protection against Phytophthora blight in pepper plants (11.8%, 18.4%, and 25.4% plants diseased, respectively). However, protective effect of the antagonist or BABA against the Phytophthora epidemic was lower than one of fungicide metalaxyl-copper.

In the plastic filmhouse test, the average percentage of plants diseased was significantly low relative to the naturally infested field (Table 3). Treatment with the antagonist strain 950923-29 and BABA in combination was most effective in suppressing the Phytophthora disease in pepper plants growing in the plastic filmhouse. The antagonist strain 950923-29, metalaxyl-copper and BABA also showed somewhat high levels of control efficacy against Phytophthora blight of pepper plants. However, there were great differences in disease incidence between replicated plots in the plastic filmhouse.

Discussion

In the present study, protective effect of the antagonistic rhizobacteria *B. cepacia* strain N9523, *Ps. aeruginosa* strain 950923-29 and DL-β-amino-n-butyric acid (BABA) against Phytophthora blight of pepper was evaluated in naturally or artificially infested fields. In general, to accomplish biological control of soilborne plant pathogens successfully, the potential antagonistic microorganisms should effectively be isolated. The potential antagonistic microorganisms selected by the *in vitro* tests often fail to effectively control plant diseases in greenhouse or field trials. Therefore, several factors such as the type, organic matter content, pH, nutrient level, and moisture content of the soil from which the potential biocontrol agent came should be considered to precisely determine protective effect of the antagonists against plant diseases (Linderman et al., 1983). The antagonistic organisms have been known to be capable of colonizing in the rhizosphere compatibly responding to the crops (Cook and Baker, 1983; Schroth and Hancock, 1981).

In many cases, biological control of soilborne plant pathogens was successfully conducted in greenhouse or fields (Linderman et al., 1983; Schroth and Hancock, 1981; Weller, 1988). Seed or root rots caused by *Pythium* spp. were effectively reduced using the antagonistic bacteria *Pseudomonas* spp.and *Bacillus* spp. (Kim et al., 1997; Moon et al., 1996; Yeom and Park, 1995) and the antagonistic fungi *Trichoderma* spp. (Hadar et al., 1984; Papavizas, 1985; Sivan et al., 1994). Diseases caused by *Phytophthora* was also biologically controlled by some mycorrhizal fungi (Marx, 1969), *Myrothecium roridum* (Gees and Coffey, 1989), *Trichoderma* spp. (Park et al., 1989) and *Gliocladium* spp. (Smith et al., 1990), *Bacillus* spp., *Streptomyces* spp., and *Pseudomonas* spp. (Ahn and Hwang, 1992; Broadbent et al., 1971; Jee et al., 1988; Kim et al., 1990; Kim and Hwang, 1992; Lee et al., 1990). In the greenhouse tests (Jee et al., 1988), suppression of Phytophthora blight of pepper by the antagonistic bacteria remained highly effective 3 weeks after application and gradually decreased thereafter. In the tests conducted in a polyethylene filmhouse, Phytophthora incidence was reduced by 64-73% using antagonists, as compared to untreated control (Lee et al., 1990). In the present study, the control efficacy of antagonist *B. cepacia* strain N9523 against Phytophthora blight of pepper was evaluated by soil-drenching with the antagonist. The protective effect was higher in soil-drench inoculation of *P. capsici* than in wounded stem inoculation (Fig. 1). The protective effect at first-branch stage was stronger than that at six-leaf stage. These data indicate that

the antagonist *B. cepacia* strain N9523 was well colonized in the rhizosphere of pepper plants to reduce the pathogen propa-gules effectively within the rhizosphere.

Phytophthora blight of pepper has been demonstrated to be suppressed by treatment with a nonprotein amino acid DL-β-amino-n-butyric acid (BABA) (Hwang et al., 1997; Sunwoo et al., 1996). A relatively high concentration of BABA at 1,000 μg/ml, which had no antifungal activity *in vitro* against Phytophthora blight with a foliar and stem spray, led to complete control of the disease (Sunwoo et al., 1996). BABA applied to the root system also protected pepper stems from *P. capsici* infection. Tomato plants at six- to seven-leaf stages sprayed with BABA protected against a challenge infection with *P. infestans* (Cohen, 1993). A single foliar spray (19.4 mM, 2,000 ppm) applied either before or after inoculation provided more than 95% control of the disease, as compared with unsprayed, challenge plants.

The treatment with BABA inhibited more pronouncedly Phytophthora infection on pepper plants soil-drenched with *P. capsici* than those inoculated by stem wounding. (Fig. 1). BABA reduced Phytophthora incidence more strongly than the antagonistic bacteria, indicating that the control efficacy of BABA was due to the induction of resistance.

Application of *B. cepacia* granules into soil provided better suppression of Phytophthora blight on red-pepper seedlings, as compared to direct drenching with *B. cepacia* suspensions (Park et al., 1989). Soil drenches and dipping of seedling roots with the antagonist suspensions were found to be more effective in disease suppression than the coating and dipping pepper seeds (Jee et al., 1988). In our study, Phytophthora blight was significantly reduced by the treatment with antagonist *Ps. aeruginosa* strain 950923-29 formulated with vermiculite (Table 2). More intensive researches about carrier substances of the antagonist should be conducted to increase the protective effect against Phytophthora blight of pepper.

In the artificially infested field test, protective effect of the antagonist *B. cepacia* was not found in the plots 20 days after inoculation of *P. capsici* (July 28), except for BABA- and metalaxyl-treated plots (Table 1). In this test, firstly, the higher concentration of inoculum applied in the plots might induce Phytophthora epidemic. Secondly, when treated with cultures of the antagonist *B. cepacia* strain N9523 2 days before inoculation of *P. capsici*, the culture components might be available as nutrients for growth of *P. capsici*. The protective effect was lower with treatment of the antagonist *B. cepacia* strain N9523 and BABA in combination than in treatment with BABA. It seems likely that BABA may be significant in inducing resistance to Phytophthora blight.

In the naturally infested field test, the protective effect was significantly high in the treatment with BABA (Table 2). In contrast to the artificially infested field test, the protection was higher by treatment with the antagonist *Ps. aeruginosa* strain 950923-29 than by BABA treatment. These results indicate that the antagonist effectively colonized the rhizosphere of pepper plants, thus leading to the high control efficacy of Phytophthora blight. Treatment with the antagonist and BABA in combination showed a synergistic effect on the protection against Phytophthora blight.

Interestingly, in the naturally infested filmhouse, the protection of pepper plants against Phytophthora blight was stronger by treatment with the antagonist *Ps. aeruginosa* strain 950923-29 and BABA than that by treatment with the matalaxyl-copper (Table 3). However, because the data of the replicates within each treatment significantly varied except for the control plot, further precise filmhouse tests will be required to obtain more reliable results.

In conclusion, antagonistic rhizobacteria *B. cepacia* strain N9523 and *Ps. aeruginosa* strain 950923-29 and a nonprotein amino acid, DL-β-amino-n-butyric acid are the effective agents capable of protecting pepper plants against Phytophthora blight. However, more detailed studies are required to elucidate formulations of antagonistic rhizobacteria for more successful protection against Phytophthora blight.

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