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Control of Fungal Diseases with Antagonistic Bacteria, *Bacillus* sp. AC-1

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ABSTRACT : Biological control of important fungal diseases such as *Phytophthora* blight of red pepper, gray mold rot of vegetables, and powdery mildew of many crops was attempted using an antagonistic bacterium, *Bacillus* sp. AC-1 in greenhouses and fields. The antagonistic bacterium isolated from the rhizosphere soils of healthy red pepper plant was very effective in the inhibition of mycelial growth of plant pathogenic fungi *in vitro* including *Phytophthora capsici, Rhizoctonia solani, Pyricularia oryzae, Botrytis cinerea, Valsa mali, Fusarium oxysporum, Pythium ultimum, Alternaria mali, Helminthosporium oryzae,* and *Colletotrichum gloeosporioides.* Culture filtrate of antagonistic *Bacillus* sp. AC-1 applied to pot soils infested with *Phytophthora capsici* suppressed the disease occurrence better than metalaxyl application did until 37 days after treatment in greenhouse tests. Treatments of the bacterial suspension on red pepper plants also reduced the incidence of *Phytophthora* blight in greenhouse tests. In farmers' commercial production fields, however, the controlling efficacy of the antagonistic bacteria was variable depending on field locations. Gray mold rot of chinese chives and lettuce caused by *Botrytis cinerea* was also controlled effectively in field tests by the application of *Bacillus* sp. AC-1 with control values of 79.7% and 72.8%, respectively. Spraying of the bacterial suspension inhibited development of powdery mildew of many crops such as cucumber, tobacco, melon, and rose effectively in greenhouse and field tests. The control efficacy of the bacterial suspension was almost same as that of Fenarimol used as a chemical standard. Further experiments for developing a commercial product from the antagonistic bacteria and for elucidating antagonistic mechanism against plant pathogenic fungi are in progress.

Studies on antagonistic microbes against various plant pathogens have been reported frequently in foreign countries as well as in Korea since researches on biological control method began. Development and utilization of biological control agents are thought to be the most desirable means in respect of environmental conservation, safety problem, and encroached ecological system due to chemical pesticides. Because antagonistic microorganisms are also living creatures in an ecological system as parts, more reseaches should be done to use them for biological control agents. Bacteria and fungi as antagonistic microorganisms have been used in many works for the control of soil-borne diseases.

The researches on antagonistic bacteria are listed as followings: *Agrobacterium radiobacter* (27), *Pseudomonas fluorescens* (14, 19, 20, 60, 71, 72, 73), *P. putida* (34, 59, 60, 63, 64), *Bacillus subtilis* (5, 6, 46, 62, 67), *Streptomyces* sp. (12, 65), *St. genus, St. griseus* (46) are reported to be used for the control of crown gall, damping-off, take all, seedling rot (47).

In Korea, *Pseudomonas* sp. (21, 29, 55), *P. putida* (34, 53, 54, 60), *P. gladioli* (9, 48, 49), *P. fluorescens* (22, 40, 58), *P. aureofaciense* (40), *P. aureoginosa* (32), *P. cepacia* (17, 18, 23, 25, 29, 30, 39, 41, 50, 57), *Bacaillus* sp. (29, 30), *B. subtilis* (11, 61, 62), *B. polymixa* (10, 23, 61), *Streptomyces* sp. (30, 38, 61, 67) for controlling damping-off, *Fusarium* wilt, and *Phytophthora* blight. In case of antagonistic fungi, many fungal antagonists effective against *Pythium* sp., *Rhizoctonia* sp., *Sclerotium* sp. were developed to commercialized products (47) under the trade names such as F-stop, BINAB T SEPPIC, WRC-GL-21, WRC-AP-1 using *Gliocladium virens* (47), *Penicillium oxalicum* (74), *Chaetomium globosum* (6), *Pythium oligandrum* (43, 44), *Talaromyces flavus, Aspergillus niger* (68), *Sporidesmium, and Sclerotium* (1, 2, 3).

In Korea, even though many researches on *Trichoderma viride* (29, 30, 48, 49, 61, 67, 75), *T. harzianum* (8, 17, 18, 22, 23, 28, 29, 30, 39, 48, 49, 56, 57) and *Gliocladium virens* (8, 24) for controlling damping-off and seedling-rots of many crops have been carried out, no microorganism has been developed to a commercialized product yet. However, *Bacillus* sp. AC-1 (33), *Pseudomonas cepacia* (17, 18, 23, 28, 39, 59), and *Trichoderma harzianum* (23, 28, 39, 57) for *Phytophthora* blight, *Pseudomonas gladioli* (49) for *Fusarium* wilt in strawberry, and *Gliocladium virens* (24) for damping-off in sesame turned out to be effective even in field tests.

To accomplish successful pest control using biological control agents, screening of antagonistic microbes which are highly effective, safe and stable in field conditions is essential. The method of treatment and formulation of antagonistic microorganisms should be developed to maintain the proper level of microbial numbers for long periods after these agents are applied to the plants.

In this review, the possibility of using an antagonistic bacterium, *Bacillus* sp. AC-1, which is effective in the control of *Phytophthora* blight in red pepper, *Botrytis* rot in chinese chives and lettuce and powdery mildew is discussed.

Screening and characteristics of an antagonistic bacterium, *Bacillus* sp. AC-1. To screen antagonistic bacteria which can control *Phytophtora* blight, uninfected roots and surrounding soil samples were taken from the infected sites. Many bacteria showing inhibitory activity against *P. capsici* were isolated by *in vitro* test. An antagonistic bacterium identified as *Bacillus* sp. in 1986 was aerobic, endospore-forming and other characteristics are described in Table 1. For an antagonistic microbe to be useful, phytotoxicity must be checked out. *Bacillus* sp. AC-1 didn't show any phytotoxicity to various plants in soil drenching and foliar application and no change in the numbers of microorganisms was observed against resident bacteria, actinomycetes and fungi in soils. The bacteria were also proved to be safe in oral and dermal tests by animal, and fish toxicity test. The antagonistic bacterium AC-1 showed broad inhibitory spectrum against *Rhizoctonia, Helminthosporium, Phytophthora, Fusarium, Alternaria, Collectotrichum, Pythium,* although it had different activity of inhibition zone *in vitro* (Table 2).

So far, many bacterial strains effective against plant fungal pathogens have been reported. For examples, *Pseudomonas cepacia* is effective against *Alternaria, Cercospora, Fusarium oxporum,*

Table 1. Physical, chemical and biological characteristics of an antagonistic bacterium *Bacillus* sp. AC-1 against *Phytophthora capsici*

Parameters	Characteristics
Isolate	Isolated from the root of red pepper
pH	Range : 4~9, Optimum : 7.3
Temperature	Optimum : 28℃, Inanimate : 95℃ for 10 min's
NaCl tolerance (%)	6.0~8.0%
Stability during subculture	15 times
Safety- crops	Red pepper, Egg plant, Tomato, Water melon, Cucumber, Radish, Chinese cabbage, Sesame, Bean
- microbes	Bacteria, Actinomycetes, Fungi in soil
- Toxicity	Acute oral (mg/kg) (>10,000)
	Acute dermal (mg/kg) (>5,000)
	Fish (TLM : ppm) (>10)

Table 2. *In-vitro* inhibition spectrum of antagonistic bacterium *Bacillus* sp. AC-1 against various plant pathogenic fungi

Inhibition zone *	Plant pathogenic fungi
+++	*Rhizoctonia solani*
	Pyricularia oryzae
	Valsa mali
++	*Helminthosporium oryzae*
	Botrytis cinerea
	Phytophthora capsici
	Phytophthora nicotianae var. *nicotianae*
	Fusarium oxysporum
+	*Colletotrichum dematum*
	Colletotrichum gloeosporioides
	Alternaria mali
	Glomerella cingulata
	Curvularia sp.
	Pythium ultimum
	Fusarium oxysporum f.sp. *cucumerinum*

*+++ : 20~40 mm, ++ : 10~20 mm, + : 8~10 mm.

Pseudomonas fluorescens (14), against *Dresehslera, Pyricularia, Phytophthora, Pythium, Sclerotinia, Verticillium,* and *Bacillus subtilis* (5, 6, 46, 62, 67) against *Fusarium, Monilia, Nectria, Phytopthora, Pythium, Sclerotium.* etc. In Korea, it was proved that *Pseudomonas aureofaciens* is effective (40) against *Fusarium moniliforme, Pythium ultimum, Pseudomonas cepacia* (23, 41) against *F. oxysporum, Phytophthora capsici, Rhizoctonia solani,* and *Bacillus subtilis* (11, 40, 61) against *Corticium rolfsii, Phytophthora capsici, Pyricularia oryzae, Rhizoctonia solani.* However, *Bacillus* sp. AC-1 strain is unique organism which is effective against various plant fungal pathogens.

Control efficacy of *Bacillus* sp. AC-1 against *Phytophthora* blight of red pepper, *Botrytis* rot of Chinese chives and lettuce and powdery mildew of cucumber. The results *in vitro* test don't always coincide with those of *in vivo* test such as in field test. So it is necessary to perform pot test in greenhouse and field test to prove its effectiveness. Red pepper was planted in pot, where pathogen was inoculated in greenhouse. Three days after transplanting, cultured AC-1 broth was soil-drenched (50 ml/plant) and a chemical pesticide (Metalaxyl copper wp. 1,000x) was sprayed to another pot as a control (Table 3). No infection appeared after 21 days in AC-1 treated pot in contrast to pesticide sprayed pot where infection was seen after 37 days, and remarkable inhibition effectiveness, moreover, was observed compared with 100% incidence of infection in plants at non-treated control pot. In greenhouse test (Table 4), AC-1 and Metalaxyl copper wp. showed inhibition of disease development after 10 days. After 20 days AC-1 plot showed 2.5% infected plants compared with 10% infected plants in pestcide treated plot. After 40 days of treatment, almost equal effectiveness was seen to control *Phytophthora* blight in red pepper. From vinyl house tests (Table 5), AC-1 treated plot demonstrated distinguished inhibition compared with none-controlled plot and showed almost equal level of incidence of infected plants as in Metalaxyl copper wp. treated plot. Furthermore, disease occurrence after 55 days, in AC-1 treated plot was lower than that of Metalaxyl copper wp. plot at Namji in Kyoungnam province.

In other field test taken at Euisung in Kyoungbuk province and Jincheon in Chungbuk province in 1988 (Table 6), mixed observation was made. That is, in Euisung, AC-1 showed better control than Metalaxyl copper wp., but results in Jincheon was opposite. Differences are thought to result not only from environmental factors in each field but also from soil

Table 3. Incidence of Phytophthora blight of red pepper in infested pot soils drenched with the culture filtrate of *Bacillus* sp. AC-1 and metalaxyl in greenhouse

Treatments	% Incidence of infected plants at each DAT*				
	7	14	21	31	37
AC-1	0	0	0	6.3	6.3
Metalaxyl/copper WP	0	0	6.3	12.5	12.5
None (control)	12.5	37.5	56.3	87.5	100.0

*DAT : Days after application of culture filtrate of the antagonistic bacteria.

Table 4. Effect of antagonistic bacterium *Bacillus* sp. AC-1 on the control of *Phytophthora* blight of red pepper in greenhouse

Treatments	% Incidence of infected plants at each DAT*			
	10	20	30	40
AC-1	0 b	2.5 c	20.0 b	42.5 b
Metalaxyl/copper wp	0 b	10.0 b	15.0 b	40.0 b
None (control)	60.0 a	90.0 a	97.5 a	100.0 a

*DAT : Days after treatment.

Table 5. Effect of antagonistic bacterium *Bacillus* sp. AC-1 on the suppression of *Phytophthora* blight of red pepper at commercial production area in Namji

Treatments	% Incidence of infected plants at each DAT *			
	15	25	40	55
AC-1	3.8 a	3.8 b	6.8 a	10.6 b
Metalaxyl/copper WP	3.1 a	3.9 ab	8.6 a	19.6 ab
None (control)	7.8 a	11.4 a	15.4 a	24.9 ab

*DAT : Days after treatment.

Table 6. Effect of antagonistic bacterium, *Bacillus* sp. AC-1 on the suppression of *Phytophthora* blight of red pepper in the field conditions

Treatments	% Incidence of infected plants	
	Euisung	Jincheon
AC-1	13.0 (75)	26.3 (33)
Metalxyl/copper	38.4 (26)	18.3 (44)
None (control)	51.7	32.7

( ) : Control efficacy, %.

settlement ability of AC-1 strain.

Possible causes of different effectiveness in the control of *Phytophthora* blight by *Bacillus* sp. AC-1. The biggest problem in biological control agents is said that even same biological control agents don't produce consistent and satisfied results in various conditions. Generally, innate soil microbes prevent alien microbes from settling in soil. It is necessary to satisfy optimal conditions for better result. The featuring causes for lowering antagonistic microbial activity are due to changes of physical, or chemical soil condition. Consequently, in greenhouse tests where are more stable and unaffected, better results were produced. But the causes which make differences in two different fields produced are considered to depend on other factors. It is surely clear that control effect can be maintained optimized only if those causes are revealed.

According to the report for inhabitation ability in soil (Table 7), after AC-1 strain (10^7 cfu/ml) was sprayed onto soil, distribution status differential by depth was observed. From surface to 5 cm, the number of 5.7×10^5 (cuf/g root) was settled, 1.3×10^5 (cfu/g root) to 10 cm at 45 days after treatment. This result hinted that the number of AC-1 had close relationship between air and soil depth. Soil samples from field test sites were collected at 7, 12, 19, 24 days after treatment, respectively, for tests on settlement ability. Eusiung samples showed higher numbers of AC-1 from surface to 5 cm even after 24 days (Table 8). This fact should be paid attention because different distribution of AC-1 which was shown from region to region may explain differences.

Soil in Euisung area was clay loam, and it contained higher amounts of Fe^{2+}, Mn^{2+}, and organic matter (2.9%). The lattest is higher than 1.1% and 0.7% in Jincheon and Koesan

Table 7. Establishment of antagonistic bacterium, *Bacillus* sp. AC-1, in the rhizosphere soil of red pepper at various depth

Depth (cm)	No. of colonies (10^5 cfu/g root)*
0~5.0	5.7
5.1~10.0	2.6
>10.0	1.3

*The number of colonies was investigated 45 days after application of *Bacillus* sp. AC-1.

Table 8. Comparison between rhizoplane and rhizosphere colonization of the antagonistic bacterium, *Bacillus* sp. AC-1 at various field soils planted with red pepper

Soil depth (cm)	Days after treatment	No. of colonies (10^7 cfu/g)					
		Euisung		Jincheon		Koesam	
		Rhizplane	Rhizosphere	Rhizoplane	Rhizosphere	Rhizoplane	Rhizosphere
0~5	7	1.3	0.05	0.7	0	0.7	0
	12	0.6	1.7	0.7	1.7	0.7	1.7
	19	0	0.7	0.3	4.0	0.3	1.7
	24	0	1.3	0.3	0	0.3	1.0
>5	7	0.7	0	0	0	0	0
	12	0	1.3	0	0	0	0
	19	0.1	0.7	0	0	0	0
	24	0	0.1	0	0	0	0
% Incidence of infected plants	Treatment	15		40		20	
	None	53		82		75	

Table 9. Physicochemical properties of soil samples collected from different regions

Regions	Inorganic ingredients (ppm)								OM	Soil	Soil
	Ca	K	Mg	Na	Cu	Fe	Mn	Zn	(%)[a]	type[b]	pH
Euisung	188	87	32	12	5	181	116	5	2.9	CL	5.1
Jincheon	171	54	24	5	3	79	32	4	1.1	L	5.2
Koesan	262	18	45	10	2	102	12	7	0.7	LS	6.3

[a]OM : Organic matter contents.
[b]CL : Clay loam, L : Loam, LS : Loamy silt.

area (Table 9). Gradual experiments were planned to along with results of physical soil analysis (Table 9). Fe and Mn contents were shown that both were not apparently linked to the growth rate of AC-1. Then the assumption is that organic matter might be the only defendable factor, and this was confirmed by the report (9, 49, 50, 66), that addition of wheat bran, barley bran, alfalfa, arrowroot leaf, and clover leaf to soil rendered antagonistic activity higher. Therefore, researches on how to provide and use organic matter with soil effciently are tasks left over which should be done for improving AC-1 activity for long-term control.

Control efficacy of *Bacillus* sp AC-1 against gray mold rot and powdery mildew. Antagonistic bacterium AC-1 has broad inhibition against various plant pathogens (Table 2). Though most of the field tests have not finished yet, those against *Phytophthora* blight, gray mold and powdery mildew were completed. In field test of gray mold which gives the most severe damage on cultivation of chinese chives and lettuce, AC-1 estabilshed control efficacy of 79.7% and 72.8% (Table 10). Meanwhile Fenarimol EC applied plot, 3 times a week to prevent powdery mildew in cucumber, showed 84.4% control efficacy, AC-1 treated plot did 83.7% (Table 11). From the relationship between incubation and control effect, it was shown that 2 days incubation after treatment had the highest control efficacy (Table 12).

Powdery mildew treated with AC-1 was observed at 1, 3, 5, 7 days after treatment (Table 13) to be found that AC-1 treatment caused 89% abnormalities of spores in comparison with 91% in Fenarimol EC treated plot. This result illustrated AC-1 treatment mutated powdery mildew spores into unharmful one. In addition, tests of controls for other genus involved with occurrence of powdery mildew such as *Erysiphe, Sphaerotheca, Leveilla,* was also turned

Table 10. Control efficacy of antagonistic bacterium, *Bacillus* sp. AC-1 treatment against gray mold rot caused by *Botrytis cinerea* in greenhouse

Vegetable	% of diseased leaf area	Control efficacy (%)
Chinese cabbage	0.1 (0.6)*	79.7
Lettuce	0.7 (2.4)	72.8

*Percent of diseased leaf area from untreated plot.

Table 11. Control efficacy of antagonistic bacterium, *Bacillus* sp. AC-1 against powdery mildew of cucumber plant

Application frequency	Control efficacy (%)	
	Culture broth of AC-1	Fenarimol*
1	16.3	39.1
2	67.4	77.2
3	83.7	84.8

*Fenarimol 12.5% EC was diluted at 1,000 fold and sprayed on cucumber shoot.

Table 12. Effect of incubation time on the activity of antagonistic bacterium, *Bacillus* sp. AC-1 against powdery mildew of cucumber

Days of incubation	Control efficacy (%)	Conc. of AC (10^7 cfu/ml)
1	64.7	3.0
2	82.3	10.4
3	78.1	4.2
5	71.4	3.0
7	62.8	7.3

Table 13. Percent of abnormally grown powdery mildew fungus, *Sphaerotheca* sp. after application of culture broth of *Bacillus* sp. AC-1 on cucumber leaves

Treatment	% of abnormally grown powdery mildew			
	1	3	5	7 DAO*
AC-1	52	89	71	69
Fenarimol**	59	91	87	85
Untreated	10	11	13	12

*DAO : Days after observation.
**Fenarimol (12.5%, EC) was diluted at 1,000 fold and sprayed on cucumber leaves.

Table 14. Genera of powdery mildew fungi inhibited by antagonistic bacterium, *Bacillus* sp. AC-1 and their host plants

Genus of powdery mildew	Host plant
Erysiphe	Radish, Tobacco, Lycium
Sphaerotheca	Rose, Pumpkin, Common burdock
Leveillula	Red pepper
Unknown	Melon, Rheum, Clover, Tall lettuce, Guridae (Angelicadahurica), Nemorosum, Dendelion, Blacked Plantain, Mugwort

out that AC-1 could control them also. When it comes to mechanisms, a few characteristics were inferred from *in vitro* test (Table 2) about its inhibition activities for *Rhizoctonia solani*, and *Phytophthora capsici*.

Microscopic observation led to these facts that AC-1 melt some mycelia of *Rhizoctonia solani* and rendered mycelium branch deformed and melted it. It seems that AC-1 controls plant pathogen by this mechanism. With powdery mildew, spores defunctioned showed plasmolysis (Table 13). Some cell walls of these spores were burst into pieces with a gray hue. This mechamism is considered as a result not from direct action of its own, but from active physiological antibiotics which is produced by AC-1.

Reports on biological control mechanism are commonly about actions by antagonistic microbes-producing antibiotics such as Agrocin (26) by *Agrobacterium*, Pyrrolnitrin (19, 25, 41), Pyoluteorin (20, 32), Phenazine carboxylic acid (14), Pendane (25), Pseudobactin (34), and Oxychlororaphine (32) by *Pseudomonas*, Bulbiformin (62), Bactirtacin (62), Bacilysin (62), and Fengymycin (62) by *Bacillus*, Gliotoxin (69, 70, 75), Viridin (4), and Trichodermin by *Trichoderma*, cochliodinol (45) by *Chaetomium*, Verrucarian (4), and Roridin (4) by *Myrothecium*, Vermistatin, Vermiculine, and Vermiaillin (14) by *Penicillium* are known. Ever without commercialized microbial pestcide of antibiotics produced by microorganism previously, possibility of using it as leading compound is thought to be important. Ways of adding high concentration, mass production and increasing amount of production are demanded for commercialization.

The characteristics and control ability of the antagonistic bacterium AC-1 against pests have been looked on. Still, many works which should have been done remain. It is hoped that this report can be a bit of help in studying useful antagonistic microbe to put microbial pesticide into commercial products.

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