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In Vivo Screening for Biocontrol Agents (BCAs) against *Streptomyces scabiei* Causing Potato Common Scab

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Through *in vitro* screening for biocontrol agents (BCAs) against *Streptomyces scabiei* causing potato (*Solanum tuberosum*) common scab, 19 streptomycete and 17 fungal isolates with antagonistic activity were selected as BCA candidates. For the selection of BCA candidates which are highly resistant to 10 kinds of antibiotics or pesticides, chemical susceptibility testing was initially performed *in vitro*. A remarkable degree of variation in susceptibility to antibiotics or pesticides was observed among the isolates tested. Streptomycete A020645 isolate was highly resistant to all the tested chemicals except neomycin up to 5,000 ppm. On the other hand, out of 36 antagonistic microbes subjected to *in vivo* pot tests using cultivar Daejima, four streptomycete isolates namely, A020645, A010321, A010564, and A020973, showed high antagonistic activity with >60% and 55% control value, respectively, and high chemical resistance to 10 kinds of chemicals. Therefore, these isolates were selected as potential BCAs for the control of potato common scab.

Keywords : Antagonistic activity, BCA, chemical susceptibility, potato common scab, *Streptomyces scabiei*

Common scab of potatoes (*Solanum tuberosum*) is caused by *Streptomyces scabiei* (Thaxter) Lambert and Loria, a very prevalent, soil-inhabiting bacterium. This serious disease can be found in all potato-growing areas throughout the world (Agrios, 1997). The scab is generally introduced with infected seed tubers. This disease drastically affects tuber quality (Hooker, 1981) due to the superficial and pitted lesions that form around the site of infection (Adams and Lapwood, 1978; Archuleta and Easton, 1981; Hooker and Page, 1960; Schroth et al., 1979). The disease causes an annual loss of several million dollars in the United States (Univ. of Illinois Ext., 1988). In Korea, the serious problem of potato scab has been observed in potato cultivation areas including Cheju island, southern Chonnam, and the alpine area of Gangwon province. In particular, Daejima which is the major potato cultivar in Korea, was found highly susceptible to the scab pathogen (Kim and Lee, 1996; Kim et al., 1998a; Kim et al., 1998b; Park et al., 2002).

Recently, there has been a rising interest in the development of environment friendly biocontrol agents (BCAs) for disease control toward sustainable agriculture. Antibiosis, or the production of antibiotics by antagonists, has been shown to play a significant role in biological control. *Streptomyces* isolates, which are effective in *in vitro* inhibition of pathogenic *S. scabiei,* have been used as BCA in the greenhouse and fields (Liu et al., 1995; Liu et al., 1996; Paulsrud, 1996; Schottel et al., 2001).

The objectives of this study were to screen BCAs against *S. scabiei* causing potato common scab, to examine their susceptibilities to 10 kinds of antibiotics or pesticides, and to determine the *in vivo* inhibitory effect of the selected BCAs on potato scab pathogen on pots.

Ten thousand microbial isolates for initial screening have been isolated from diverse soil samples in Korea using the dilution plating method. Four species of potato scab-related pathogens such as *S. scabiei* DSMZ 40962, *S. scabiei* S-67 (pathogenic, Korean isolate), *S. acidiscabies* ATCC 49004, and *S. turgidiscabies* S-27 (pathogenic, Korean isolate) were used in this study. The two Korean isolates were obtained from Dr. Chunkeun Lim of the Kangwon National University. Nineteen actinomycete and 17 fungal isolates including A000309, A020967, and F010315 were selected through the initial screening for BCA against *S. scabiei* causing potato common scab.

The antagonistic actinomycete microbes selected in this study were identified based on the International *Strepto-*

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myces Project (ISP) criteria. The fungal microbes were identified based on the morphological and cultural characteristics. The Microbial Resource Data Base (MRDB) of the Korea Research Institute of Bioscience & Biotechnology holds the isolates under lyophilized condition. The isolates were also maintained in the refrigerator (2-3°C) until use for cultivation.

The media for selective isolation and preservation of actinomycetes were Humic acid-vitamin agar (HV medium) and Bennett's agar (B medium), respectively. HV medium contains (g/l) humic acid (dissolved in 10 ml of 0.2 N NaOH), 1.0; Na_2HPO_4, 0.5; KCl, 1.7; $MgSO_4 \cdot 7H_2O$, 0.05; $FeSO_4 \cdot 7H_2O$, 0.01; $CaCO_3$, 0.02; agar, 20; each of thimine-HCl, riboflavin, niacin, pyridoxin-HCl, inositol, Ca-pantothenate, aminobenzoic acid, 0.5 mg and biotin, 0.25 mg as vitamin; cycloheximide 50 ppm; and nalidixic acid, 10 ppm (adjusted to pH 7.2 before sterilization). The composition of B medium contains (g/l) glucose, 10; yeast extract, 1; Bacto-peptone, 2; beef extract, 1; and agar, vitamin, and antibiotics (the same amount as HV medium). In addition, the actinomycete strains were first inoculated in 200 ml Erlenmeyer flasks containing 60 ml of the screening medium consisting of soluble starch, 10; glucose, 20; soybean meal, 25; beef extract, 1; yeast extract, 4; NaCl, 2; K_2HPO_4, 0.25; and $CaCO_3$ (2 g/l) (adjusted to pH 7.2 before sterilization) and cultured on a rotary shaker (150 rpm) at 28°C for 5 days. On the other hand, PGA consisting of potato (200), glucose (2), agar (15 g/l), and PD broth consisting of potato (200) and glucose (2 g/l) without agar were used for solid and liquid cultivation of fungi, respectively. For potato infusion, 200 g of scrubbed and sliced potatoes in 1,000 ml water was boiled for 1 hour and then the extract solution was passed through fine sieve.

The chemicals consisting of antibiotics and pesticides used in this study are as follows: Dazomet (tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione, GR; dimethomorph (4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl]morpholine)+dithianon (5,10-dihydro-5,10-dioxonaphtho[2,3-*b*]-1,4-dithiin-2,3-dicarbonitrile), WP); mancozeb [manganese ethylenebis(dithiocarbamate)(polymeric), P], oxadixyl [N-(2,6-Dimethylphenyl)-2-methoxy-N-(2-oxo-3-oxazolidinyl) acetamide, WP]; streptomycin sulfate (*O*-2-deoxy-2-(methylamino)-a-L-glucopyranosyl-(1->2)-*O*-5-deoxy-3-*C*-formyl-a-L-lyxofuranosyl-(1->4)-*N,N'*-bis(amino-iminomethyl)-D-streptamine, WP), validamycin A (1,5,6-trideoxy-4-*O*-β-D-glucopyranosyl-5-(hydroxymethyl)-1-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-D-*chiro*-inositol, WP); chloramphenicol [2-[(2,2-dichloroacetyl) amino]-3-hydroxy-3-(4-nitrophenyl) propyl ester, tablet], cycloheximide ([1*S*-[1a(*S**),3a,5β]]-4-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione); nalidixic acid(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid, solution); and neomycin sulfate (solid). All concentrations (ppm) were adjusted to 1-10,000 ppm based on the active ingredients. All chemicals were purchased from the Sigma-Aldrich Chemical Co. or supplied by KRICT, Korea.

The stock solution (1 ml/100 ml medium) containing cells of potato scab pathogen, *S. scabiei,* was added and mixed before pouring on Bennett's agar. Paper disc and streak-inoculation methods were used for the initial antimicrobial activity determination using broth cultures or solid cultures. The supernatant of culture broth was used for the paper disc method, and each antagonistic cell was taken from the colony on solid stock plates using loop and then inoculated on test plates by the streaking method.

To control target disease together with the chemicals, as well as to prevent reduction in antagonistic activity in the field contaminated or over-treated by lots of chemicals for a long time and easily trace antagonistic microbes treated in the soil, the chemical susceptibility level of antagonistic microbes to antibiotics or pesticides were examined *in vitro*. The susceptibility testing on *S. scabiei* was done by means of agar screening method on media as above. The solution (100 ul) containing scab pathogen was spread on Bennett's agar (for streptomycete isolates) or potato glucose agar plates (for fungal isolates). Paper discs treated with antibiotics and pesticides adjusted to different concentrations in the range of 1-10,000 ppm were placed on the agar surface. The plates were incubated for 48 hours at 28°C. The minimal inhibition concentration (MIC) of antibiotics and pesticides against *S. scabiei* was determined by colony growth on media. Growth of single colony was taken as indication of resistance. Chemical-free plates were used as growth control.

For the *in vivo* pot test, the active isolates were cultured in 1 l Erlenmeyer flasks containing 200 ml of the same medium as above. Each culture was freeze-dried and finely ground before formulation. The freeze-dried cultures were mixed with ingredient consisting of zeolite 30, bentonite 10, glucose 2, soybean meal 2, and chitin 2 g/200 ml culture. The 2 g of powdered formulants (CFU, $>1 \times 10^9$) were treated four times during 4 months in and on soil of pots (three replicates) using a small spoon. Qualified seed tubers of cultivar Daejima were planted at 10 cm deep in pots loaded with vermiculite-based root medium. Control value range (0-100%) was evaluated by the mean percent score of inhibition based on the number of scab and lesion area formed on tuber on pots (350 cm^2) in comparison with control at 4 months after treatment. Control pots were treated with scab pathogen of the same form of the powdered formulant (positive control) or with the ingredients without scab pathogen (negative control).

Through *in vitro* screening of antagonistic isolates against

Table 1. List of antagonistic streptomycete and fungal isolates against *S. scabiei* causing potato scab

Isolate no.	Inhibition zone*	Isolate no.	Inhibition zone
F010315	16	A010321	16
F010418	15	A010564	9
F010592	8	A020645	19
F010608	18	A020973	25
F010664	20	A020069	19
F020232	12	A020447	8
F020233	8	A021194	16
F020352	18	A91199	34
F020469	16	A92089	12
F020486	21	A92113	26
F010080	19	A92217	20
F010218	17	A92202	27
F010624	15	A92345	15
F020460	12	A000519	13
F020508	11	A001280	31
F020524	24	A020246	ND
F020543	21	A020337	17
A000309	8	A020967	30

*Inhibition zone (diameter, mm) was measured by paper disc method 5 days after treatment.

S. scabiei causing potato common scab using paper disc and streak-inoculation methods, 19 streptomycete and 17 fungal isolates with antagonistic activities were selected as BCA candidates. The antagonistic activity of streptomycete isolates was more active to *S. scabiei* than to fungal isolates. Three streptomycete isolates including A91199 were very active against *S. scabiei*, representing >30 mm inhibition zone. In addition, three fungal isolates including F020524 were active against *S. scabiei*, representing >20 mm inhibition zone (Table 1).

The chemical susceptibility of 36 antagonistic isolates and 3 scab pathogens including *S. scabiei*, *S. acidiscabies*, and *S. turgidiscabies* to 10 kinds of antibiotics or pesticides including dazomet, mancozeb, streptomycin, and chloramphenicol were evaluated by agar screening method. A remarkable degree of variation in susceptibility to antibiotics or pesticides was observed among the antagonistic isolates and scab pathogens tested (Tables 2, 3). The streptomycete isolates including A91199, A020967, A92113, and A92217 were susceptible to dazomet, mancozeb, nalidixic acid, and neomycin at 50-100 ppm, while A010137 and A020645 were resistant to most of the antibiotics tested at 5,000 ppm. *S. scabiei* ATCC 40962 was highly susceptible to all the

Table 2. Susceptibility of antagonistic streptomycete isolates and potato scab pathogens to chemicals

Isolate no.[b]	MIC									
	Daz[a]	Dd	Man	Oxa	Str	Val	Chl	Cyc	Nal	Neo
A000309	1000	>5000	100	100	100	2000	100	>5000	500	100
A010321	1000	>5000	1000	1000	1000	2000	100	>5000	50	50
A010564	1000	>5000	100	500	1000	2000	500	>5000	1000	1000
A020645	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>1000
A020973	1000	>5000	100	100	500	5000	50	>5000	500	500
A020069	1000	>5000	500	2000	500	5000	500	>5000	500	500
A020447	1000	>5000	100	100	100	2000	100	>5000	100	100
A021194	1000	>5000	500	1000	500	5000	100	>5000	500	500
A91199	50	>5000	100	100	100	500	100	>5000	50	50
A92089	500	2000	500	500	5000	1000	2000	>5000	50	500
A92113	100	5000	100	100	2000	500	500	1000	50	50
A92217	100	1000	50	50	1000	1000	100	5000	50	50
A92202	500	>5000	100	100	2000	1000	1000	>5000	500	50
A92345	500	5000	1000	1000	>5000	5000	500	>5000	50	100
A000519	1000	1000	1000	1000	50	1000	100	>5000	500	50
A001280	500	>5000	100	500	5000	2000	500	>5000	50	50
A020246	500	5000	50	50	1000	2000	1000	>5000	100	50
A020337	500	5000	100	100	100	2000	100	>5000	50	100
A020967	50	10	100	100	1000	1000	1000	>5000	50	50
S-27	2000	>5000	5000	5000	2000	>5000	1000	>5000	500	1000
S-67	5000	1000	>5000	100	1000	2000	5000	>5000	50	50
ATCC49004	2000	>5000	1000	1000	2000	500	100	>5000	1000	50
DSMZ40962	50	10	50	10	50	500	50	1000	100	10

[a]Daz, dazomet; Dd, dimethomorph+dithianon; Man, mancozeb; Oxa, oxadixyl; Val, validamycin A; Chl, chloramphenicol; Cyc, cycloheximide; Nal, nalidixic acid; Neo, neomycin; Str, streptomycin.

[b]S-27, *S. turgidiscabies* (Korean isolate); S-67, *S. scabiei* (Korean isolate); ATCC49004, *S. acidiscabies*; DSMZ40962, *S. scabiei*.

Table 3. Susceptibility of antagonistic fungal isolates to chemicals

Isolate no.	MIC									
	Daz[a]	DD	Man	Oxa	Str	Val	Chl	Cyc	Nal	Neo
F010315	>5000	>5000	500	2000	>5000	>5000	>5000	2000	>5000	>5000
F010418	500	>5000	2000	5000	>5000	>5000	>5000	2000	>5000	>5000
F010592	100	1000	500	1000	>5000	>5000	>5000	500	>5000	>5000
F010608	1000	5000	500	500	>5000	>5000	>5000	2000	>5000	>5000
F010664	2000	>5000	500	1000	>5000	>5000	>5000	2000	>5000	>5000
F020232	>5000	1000	500	500	>5000	>5000	>5000	500	>5000	>5000
F020233	500	2000	500	500	>5000	>5000	>5000	1000	>5000	>5000
F020352	500	1000	500	500	>5000	>5000	>5000	2000	>5000	>5000
F020469	>5000	5000	500	500	>5000	>5000	>5000	500	>5000	>5000
F020486	2000	>5000	1000	500	>5000	>5000	>5000	1000	>5000	>5000
F010080	500	>5000	1000	1000	>5000	>5000	>5000	2000	>5000	>5000
F010218	2000	1000	1000	1000	>5000	>5000	>5000	1000	>5000	>5000
F010624	500	>5000	100	500	>5000	>5000	>5000	2000	>5000	>5000
F020460	100	2000	100	500	>5000	>5000	>5000	500	>5000	1000
F020508	500	5000	100	100	>5000	>5000	>5000	100	>5000	1000
F020524	5000	5000	1000	>5000	>5000	>5000	>5000	>5000	>5000	>5000
F020543	100	1000	100	100	>5000	>5000	>5000	1000	>5000	>5000

[a]Daz, dazomet; Dd, dimethomorph+dithianon; Man, mancozeb; Oxa, oxadixyl; Val, validamycin A; Chl, chloramphenicol; Cyc, cycloheximide; Nal, nalidixic acid; Neo, neomycin; Str, streptomycin.

tested antibiotics except cycloheximide and validamycin in the range 10-100 ppm. A streptomycete A020645 isolate was highly resistant to all the tested antibiotics except neomycin up to 5,000 ppm (Table 2). Interestingly, an aminoglycoside antibiotic, neomycin, was highly active against streptomycete strains, showing its potential as a selective natural metabolite for the control of *S. scabiei* causing potato scab. On the other hand, three fungal isolates, F010592, F020460, and F020543, were susceptible to dazomet at 100 ppm, while four fungal isolates, F010315, F010232, F020469, and F020524, were resistant up to 5,000 ppm level (Table 3). This information may have important implications in evaluating the potential of the BCA in adapting to the potato field. For BCAs to survive and continue to multiply causing control in the field, antibiotic-resistant BCAs should be screened and selected.

In this study, 36 antagonistic microbes were subjected to *in vivo* pot tests using cultivar Daejima. The results of *in vitro* antagonistic activity of microbes tested differed from those *in vivo* pots, depending on the isolates. As shown in Table 4, most of the streptomycete isolates were more active to potato scab than fungal isolates. Out of 36 antagonistic microbes subjected to *in vivo* pot tests, six streptomycete isolates including A020645 and A010321 showed >40% control value. Four streptomycete isolates such as A020645, A010321, A010564, and A020973 showed high inhibitory activity with >60% and 55% control value, respectively, against scab pathogen, and high chemical resistance to antibiotics or pesticides. These six isolates were selected as potential BCAs for the control of potato scab.

Results of this study showed that the formulants containing freeze-dried cell masses and metabolites were effective at controlling potato scab. In particular, freeze-dried cultures of non-pathogenic streptomycete strains were more effective than fungal isolates as shown in Table 4.

Control of common scab of potato has been through the use of certified scab-free seed potatoes or through a chemical

Table 4. *In vivo* activity of BCAs against potato scab

Isolate no.	Control value*
A020645	65
A010321	60
A010564	60
A020973	55
A020413	40
A000309	40

*Control values were evaluated by the mean percent score of inhibition based on the number of scab and lesion area formed on tuber (cultivar Daejima) in pots 4 months after treatment. >10% scab lesion area observed (no inhibition, score 0); 5% scab lesion area observed (= very slight inhibition, score 20); more or less scab observed (slight inhibition, score 40); small number of scab observed (somewhat inhibition, score 60); just a few scab observed (very considerable inhibition, score 80); no scab observed (complete inhibition, score 100). Control values below mean score 40 were omitted in this table.

seed treatment with pentachloronitrobenzene (PCNB) or with maneb-zinc dust (Agrios, 1997). Mancozeb has been used as a preventive chemical for the disinfection of seed potatoes. A soil fumigant, dazomet (Basamid®), has also been used for the control of potato scab, as well as soil-borne fungi, soil insects, a wide range of weeds, and nematodes in Korea. However, the *in vitro* antibiotic activity of dazomet and mancozeb against scab pathogens, except *S. scabiei* DSMZ 40962, were not so high, although they showed more inhibitory effect on fungal isolates than streptomycete isolates. Interestingly, an aminoglycoside derivative, neomycin, was highly active against most of the streptomycete isolates including antagonistic microbes or scab pathogens. Generally, the >60% control values obtained after treatment with only BCAs without any application of existing chemicals in the field are considered high in comparison with those of previous BCAs. Further research work on the development of effective formulation and fitness of selected BCAs in soil are now ongoing.

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