Biological control of *Botrytis cinerea* on tomato using antagonistic bacteria

Sung-Jun Hong[1], Yong-Ki Kim[2], Hyeong-Jin Jee[3], Jong-Ho Park[4], Eun-Jung Han[5], Nan-Hee An[6], Jung-Hyun Kim[7], Hyung-Jin Goo[8]

Key words: Biological control, *Botrytis cinerea*, antagonistic bacteria

Abstract

Botrytis cinerea *infects stems and leaves of greenhouse tomatoes and can cause serious economic losses. This study was conducted to develop environment-friendly control method against tomato gray mold. Antagonistic microorganisms (bacteria) were screened for control activity against* Botrytis cinerea, *both in vitro and in vivo, using stem sections. One hundred bacterial strains were isolated from the rhizospheric soil of various plants including tomato. These strains were screened for growth inhibition of* Botrytis cinerea *on agar plate by the dual culture and thirty strains showing strongly inhibitory effect against the pathogen were selected first. Among thirty strains, JB 5-12, JB 22-2, JB 22-3, U 4-8 and U46-6 reduced significantly disease incidence, when applied simultaneously with the pathogen. These results suggested that five antagonistic bacteria strains selected have the potential to control tomato gray mold in organic farming.*

Introduction

Botrytis cinerea is a well-known plant pathogenic fungus with a wide host range that causes heavy yield losses in tomato. The fungal pathogen infects stems, flowers and fruits by direct penetration or through wounds caused by cultivation practices. Fungicides are the primary strategies to control gray mold of tomato. By contrast, chemical control may have several side effects, including the development of resistant strains and environmental contamination. Synthetic fungicides are gradually becoming ineffective. Consequently, consumer concerns and regulatory restrictions over pesticide residues on foods have emphasized the need for replacing synthetic chemicals with other methods for gray mold control. Biological control using natural antagonistic microorganisms has been extensively studied, and some fungi and bacteria have been demonstrated to be effective against gray mold. In the present paper, the objectives were to (i) survey a collection of antagonistic bacteria isolated from various origins and antagonistic bacteria strains selected for their potential

[1] Organic Agriculture Division, National Academy of Agricultural Science, RDA, Suwon, 441-707, Republic of Korea, E-Mail: hongsj7@korea.kr, Internet www.naas.go.kr

[2] As above

[3] As above

[4] As above

[5] As above

[6] As above

[7] As above

[8] *Gimpo Agricultural Technical Center, Gimpo 415-743, Korea*

biocontrol activity in controlling gray mold of tomatoes, and (ii) investigate the efficacy of antagonistic bacteria strains selected in reducing gray mold in tomatoes.

Materials and methods

Isolation and screening of antagonistic bacteria strains(*In vitro*)

Bacterial strains were isolated from the rhizospheric soil of various plants including tomato. All 100 bacteria strains isolated were initially screened *in vitro* to assess their antagonistic ability against *Botrytis cinerea* based on the modified methods of dual cultures. Dual cultures were started with bacteria strains and the pathogen placed 4 cm apart on PDK plates(9 cm diameter). Plates were incubated in a growth chamber at 20℃. After seven days, the inhibitory effect of antagonistic bacteria strains was evaluated considering the ability of the bacteria strains to reduce the pathogen mycelium growing.

In vivo biological control activity

The *in vivo* antifungal activity of bacterial metabolites was investigated using stem section bioassays. The method of stem section bioassay was as follows : the terminal ends of stem sections, 40mm long, were dipped to a depth of 5mm in a *Botrytis* spore suspension(1×10^5 spore/ml), and air-dried for 1-2h. The terminal ends were then dipped in a suspension of antagonistic bacteria strains. Treated sections were placed three or four per petri-dish(unmoistened) and incubated in dry sealed boxes. The stem sections were incubated in a controlled temperature incubator(15℃) without lighting. The percentage of infected stem sections was assessed after 11-15 days.

Identification of antagonistic bacteria strains

Antagonistic bacteria strains were identified by PCR amplification and partial sequencing of the 16s ribosomal DNA(rDNA), using the primers 8F and 1492R.

Results

Screening of antagonistic bacteria strains(*In vitro*)

One hundred bacterial strains were screened for growth inhibition of *Botrytis cinerea* on agar plate by the dual culture and thirty strains showing strongly inhibitory effect against the pathogen were selected first(data not shown).

In vivo biological control activity

Among thirty strains, JB 5-12, JB 22-2, JB 22-3, U 4-8 and U46-6 reduced significantly disease incidence, when applied simultaneously with the pathogen. (Tab. 1).

Tab. 1: Effect of Antagonistic bacteria on *Botrytis cinerea* using stem section bioassays.

Bacteria strains	Disease incidence (%)			original suspension density(cfu/ml)
	original suspension	10× dilution suspension	100×dilution suspension	
B 5-12	0	0	0	5.0×10^{7}
JB 2-9	5.6	66.7	66.7	7.5×10^{6}
JB 8-8	0	66.7	77.8	2.8×10^{7}
JB 22-2	0	0	5.6	1.2×10^{8}
JB 4-7	11.1	55.6	44.4	1.2×10^{7}
JB 24-11	0	55.6	50.0	3.1×10^{7}
JB 37-2	27.8	61.1	61.1	3.1×10^{7}
JB 22-1	27.8	44.4	66.7	2.1×10^{7}
JB 11-8	0	88.9	77.8	2.1×10^{7}
JB 23-5	0	11.1	33.3	9.7×10^{7}
JB 24-5	27.8	50.0	77.8	2.6×10^{7}
JB 8-11	27.8	77.8	55.6	1.2×10^{7}
JB 22-3	0	0	0	1.8×10^{7}
JB 5-2	22.2	77.8	72.2	4.2×10^{6}
88-7-2	0	88.9	72.2	1.3×10^{7}
U 46-6	0	0	0	3.5×10^{7}
NH 31-5	5.6	61.1	66.7	1.1×10^{6}
U 4-8	0	0	0	5.8×10^{7}
EH 23-5	5.6	27.8	77.8	2.5×10^{7}
Y 6-5	0	38.9	66.7	9.7×10^{7}
K 39-10	0	50.0	66.7	1.9×10^{7}
MH 40-2	0	88.9	55.6	2.6×10^{7}
KH 38-1	0	27.8	77.8	4.0×10^{7}
KH 32-6	0	61.1	50.0	3.2×10^{7}
CNB-2	0	27.7	22.2	4.1×10^{7}
CNB-3	16.7	38.8	88.8	4.1×10^{7}
OMC	0	44.3	55.5	2.4×10^{7}
EHR	0	50.0	72.2	7.6×10^{7}
SEC	0	61.0	66.7	3.2×10^{7}
SEB	16.7	27.7	50.0	8.5×10^{7}
fungicide	0			
control	88.9			

Identification of antagonistic bacteria strains

According to 16S rDNA sequence data, five antagonistic bacterial strains were identified as *Pseudomonas chlororaphis, Pseudomonas* sp., *Bacillus amyloliquefaciens, Pseudomonas fluorescens, Streptomyces* sp.(Tab. 2).

Tab. 2: Identification of five antagonistic bacterial strains against *Botrytis cinerea* according to the Sequence similarity of 16S rDNA

strains	Identification	Similarity(%)
JB 5-12	*Pseudomonas chlororaphis* subsp. *aurantiaca*	99
JB 22-2	*Pseudomonas* sp.	99
JB 22-3	*Bacillus amyloliquefaciens*	99
U 46-6	*Pseudomonas fluorescens*	99
U 4-8	*Streptomyces* sp.	99

Discussion

Biological control has been considered as one of most promising alternatives to chemical fungicides, which employs antagonistic bacteria to protect fruits and vegetables from infection by phytopathogens. In this study, we isolated and identified three *Pseudomonas* sp. strains, one *Bacillus amyloliquefaciens,* one *Streptomyces* sp. from the rhizospheric soil of various plants. *In vitro* activity analysis indicated that these strains produced diffusible antifungal compounds. The stem section bioassay demonstrates the ability of five antagonistic bacterial strains(JB 5-12, JB 22-2, JB 22-3, U 46-6, U 4-8) to control *Botrytis* infecting wounded stem tissue. Also, five antagonistic bacterial strains maintained strongly inhibitory effect against *Botrytis cinerea* at 100 time dilution suspension. These results indicate that five antagonistic bacteria strains selected could be developed as microbial agents for the control of Botrytis diseases. Accordingly, further studies are required for mass-production and formulation for commercialization.

Conclusions

Our data showed that five antagonistic bacterial strains had potential biocontrol activity against gray mold caused by *Botrytis cinerea* in tomato.

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