



The Biocontrol Effect of Trichoderma and Bacillus Subtilis SY1

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Abstract

As years of unreasonable farming practices in agriculture soil damaged seriously. The soil-borne disease and the chemical residue are two serious problems of soil pollution which affect the yield and quality of agricultural products. Ecological remediation of soil is an effective way to resolve these problems. In this paper, the agents' trichoderm and the Bacillus subtilis SY1 were used to improve the ecosystem function and reduce the disease occurrence.

Keywords: Biocontrol effect, Trichoderma, Bacillus subtilis SY1

1. Introduction

The sensitivity of Ginseng to pathogenic fungus has increased after being domesticated out of wildness state. It is reported that there are more than thirty kinds of diseases harmful to Ginseng's growth statistically which can aggrieve its roots, flowers, fruits and could result in fall of production, quality of seeds and roots (Charron.D., Gagnon. D., 1991; W. G. Bailey. 1996.).

Except for the chinese herbal medicine, the problem of plant fungal pathogens are common in agriculture nowadays, especially in facility agriculture. As a new direction for biological control method has recently been developed and beneficial agents, such as Bacillus subtilis (Shinji Mizumoto et al, 2007; Wang J et al, 2007; Marc Ongena et al, 2005) are widely used.

In this paper, trichoderm and Bacillus subtilis SY 1 were used in the experiments to determine their biocontrol effect to pathogen fungi in soil. trichoderm a could antagonize several typical pathogen fungi of American ginseng and decrease the disease occurrence. The eggplant planting experiments indicated that Bacillus subtilis SY1 not only could antagonize several pathogen fungi of eggplant but could promote the seedling growth and increase its stress tolerance.

2. Materials and method

2.1 Microbial agents

The Bacillus subtilis SY1 used through this work was preserved in the laboratory.

Nine pathogenic fungi of American Ginseng and one anti-biotic fungus *Trichoderma* were separated in the Ginseng planting base.

The four vegetable plant pathogens are provided by Vegetable Research Institute, Tianjin Academy of Agriculture Sciences.

2.2 Experimental method

2.2.1 American ginseng experiment

2.2.1.1 The confrontation experiment

In the confrontation experiment, on the PDA media a piece of 5 mm diameter antagonistic fungi (3 days old cultures) was put on one side and the pathogen fungi (7 days old cultures) of the same size was put on the other side, 4cm apart from each other. In the control experiment 5mm diameter pathogen fungi were put on the PDA media. All the experiments were repeated 5 times The plates were incubated at $28\pm 1^\circ\text{C}$ for 7 days to detect the antibacterial effect.

$$I = \frac{R_2 - R_1}{R_2} \times 100\% \quad (1)$$

I—Antibacterial effect;

R1—Straight-line distance between the center of the pathogen fungi and the edge of antagonistic fungi;

R2—The radius of the control pathogen fungi.

2.2.1.2 American ginseng field experiment

The field experiment of inoculated and control were undertaken in the American ginseng planting base. The inoculated and the control were undertaken simultaneously and were both repeated three times. After disinfecting, the ginseng roots were regularly arranged on the land which surface soil was removed in advance. After covering the soil back onto ginsengs, 5 mL trichoderm suspension was added as liquid to the roots of the American ginseng. Three times of inoculation were did in the first three month after sowing. The disease occurrence were determined after 12 month.

2.2.2 Eggplant experiment

2.2.2.1 Antagonistic examination

In vitro antagonistic examination of the antifungal activity of *Bacillus subtilis* SY1 was tested against the four typical soil-borne pathogen diseases on the PDA media. Spore suspensions with different fungi (5 days old cultures, the concentration was more than 106 cfu / mL) have been prepared in 0.85% sterilized saline. The melted solid wateragar medium was put on the plates, the sterile stainless steel columns were put on the frozen solid agar. When the melted semisolid PDA media cooled to $40\text{-}50^\circ\text{C}$, 1 mL of spore suspensions of each fungus was put in and well-mixed with PDA media. The mixture was then put onto the wateragar medium plates using transfer pipet. When the agar was frozen solid, the stainless steel columns were taken away and 50 μL transformation was put into the hole except the controls (put into 50 μL sterilized saline). Every treatment was repeated three times. The plates have been incubated at $28\pm 1^\circ\text{C}$ for 7 days to detect the diameter of fungal inhibition around.

2.2.2.2 Eggplant field experiment

2.2.2.3 Eggplant seedling growth promoting examination

Eggplant seeds were disinfected with 70% ethanol -water ratio solution and then with 0.5% sodium hypochlorite. After rinsing six times with sterile water, the seeds were soaked in the water to pregermination. After three days, eggplant seeds were selected and sown into the pots. The plants were irrigated with water every other day. After 20 days, the growth situation and antioxygen enzyme of eggplant seedling were determined.

2.3 Analysis method

Chlorophyll content—By means of ethanol extraction

Superoxide dismutase (SOD)—By means of nitroblue tetrazolium photoreduction

Catalase (CAT) —By means of ultraviolet absorption

Peroxidase (POD) —By means of guaiacol method (Wu et al, 2006)

3. Results and discussion

3.1 American ginseng experiment

3.1.1 The confrontation experiment

3.1.2 American ginseng field experiment

The American ginsengs in each block were dug out to be detected one years later, and the root diseases were classified

according to its condition. Table 2 shows the ginseng root diseases rate.

root diseases classification:

Grade 0: ginseng root anosis

Grade 1: small disease spots on the ginseng root surface

Grade 2: disease spots on 1/5 of the surface

Grade 3: disease spots on 1/5 of the surface

Grade 4: 1/2 of the root rot

Grade 5: most of the root rot

$$\text{Disease rate \%} = 100 - \frac{\text{Grade 0 ginseng number}}{\text{total ginseng number}} \times 100 \quad (2)$$

3.2 Eggplant experiment

3.2.1 The antagonistic examination

In vitro antagonistic examination of the antifungal activity of *Bacillus subtilis* SY1 was tested against the four typical soil-borne pathogen diseases on the PDA media. Spore suspensions with different fungi (5 days old cultures, the concentration was more than 10^6 cfu / mL) have been prepared in 0.85% sterilized saline. The melted solid water agar medium was put on the plates, the sterile stainless steel columns were put on the frozen solid agar. When the melted semisolid PDA media cooled to 40-50°C, 1 mL of spore suspensions of each fungus was put in and well-mixed with PDA media. The mixture was then put onto the water agar medium plates using transfer pipet. When the agar was frozen solid, the stainless steel columns were taken away and 50 µL transformation was put into the hole except the controls (put into 50 µL sterilized saline). Every treatment was repeated three times. The plates were incubated at 28±1°C for 7 days to detect the diameter of fungal inhibition around.

From Table 3 and Fig 1 we can see that *Bacillus subtilis* SY1 has antifungal activity to all these four pathogens to a certain extent, especially *D. Alternaria solani* the diameter of fungal inhibition ring attained 43.6 mm. *Alternaria solani* is the most common and harmful soil-borne pathogen fungi in agriculture which causes decline of plant yield and quality every year. Bioremediation of inoculating beneficial bacteria *Bacillus subtilis* SY1 is an effective method to decrease the pathogen fungi amount. The lower quantity and activity of soil-borne pathogen fungi could decrease the disease occurrence so as to guarantee a bumper harvest.

3.2.2 Eggplant field experiment

The eggplants growth condition were determined after 3 months, and the diseases condition were classified. Table 2 shows the eggplants diseases rate.

disease investigation:

Grade 0: plant anosis

Grade 1: few small disease spots on the leaves and the stems anosis

Grade 2: disease spots on most of the leaves and the stems anosis

Grade 3: most of the leaves turn yellow but the stems anosis

Grade 4: most of the leaves turn yellow and the stems rot

Grade 5: withered plant

$$\text{disease index \%} = \frac{\sum(\text{disease plant numbers of each grade} \times \text{grade number})}{(\text{total plant numbers} \times 9)} \times 100 \quad (3)$$

3.2.3 The growth situation and antioxidant enzyme of eggplant seedling

After 20 days' growth, eggplant seedlings selected in random order were dug out and cleaned with sterile water and plant height, dry weight, chlorophyll content, antioxidant enzyme such as SOD, CAT and POD were determined. The results are shown in Table 2.

From Table 2 we can clearly see that the growth of seedling and the antioxidant enzyme activities in the plant all improved after inoculating the *Bacillus subtilis* SY1. The Plant height and the dry weight of the seedling increased 56.61 % and 33.55 % respectively. The improvement of chlorophyll content and antioxidant enzyme activities stands for the increase of plant metabolism and stress tolerance. The chlorophyll content is closely related with photosynthesis which provide energy to the plant. SOD is a common enzyme that exists throughout the animal and plant kingdoms, it could remove the superoxide radical. The activity of CAT has a great impact on plant resistance to cold and disease. POD is a highly active enzyme that also common exists in the plant which is closely related to the respiration, photosynthesis and degradation reaction of growth hormone.

4. Conclusions

In the American ginseng planting experiment the biocontrol agent trichoderma has great antifungal effect to several pathogens that common in American Ginseng planting soil. After inoculating the trichoderma, the disease occurrence decreased significantly.

In the eggplant planting experiment the *Bacillus subtilis* SY1 has great antifungal effect on pathogens and the growth and stress resistance of the seedlings in the inoculated soil increased. After inoculating, the plant height, dry weight and chlorophyll content increased by 56.61%, 33.55% and 40.1% respectively. The antioxidant enzymes SOD, CAT and POD improved significantly by 103.2 %, 127.3% and 81.5 %.

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Table 1. Antibacterial effect of trichoderma to American ginseng fungous diseases

	Pathogenic fungi	R ₁ /cm	R ₂ /cm	I= (R ₂ -R ₁)/R ₂ ×100%
F ₁	<i>Alternaria panax</i>	2.5	9	72.2
F ₂	<i>Fusarium sp.</i>	1.5	5	70.0
F ₃	<i>Phytophthora cactorum</i>	1.7	9	81.1
F ₄	<i>Cylindrocarpon sp.</i>	2	9	77.8
F ₅	<i>Acremoniella cucurbitae</i> <i>Schulzlet Saccl</i>	2.0	7	71.4
F ₆	<i>Erysiphe panacis</i> Bai et Wang	1	4	75.0
F ₇	<i>Monilia cinerea</i> Bon	2	8	75.0
F ₈	<i>Botrytis cinerea</i> Persl	2.6	9	73.3
F ₉	<i>Phoma panacicola</i> Nakata et <i>Takimoto</i>	2.2	8	72.5

Table 2. The disease rate of each block

		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Zero-grade rate %	Disease rate%
Block: 1	control	9	16	3	0	0	0	32.14	67.86
	inoculated	17	12	0	0	0	0	58.62	41.38
Block: 2	control	9	15	3	0	0	0	33.33	66.67
	inoculated	13	14	0	0	0	0	48.15	51.85
Block: 3	control	7	19	4	0	0	0	23.33	76.67
	inoculated	16	12	0	0	0	0	57.14	42.86

Table 3. Inhibitory activities of *Bacillus subtilis* SY1 to four typical pathogens

	Pathogenic bacteria	Diameter of fungal inhibition ring (mm)
A	<i>Pythium aphanidermatum</i>	22.4
B	<i>Fusarium oxysporum f.sp.lycopersici</i>	20.2
C	<i>Botrytis cinerea Pers.</i>	16.5
D	<i>Alternaria solani</i> (Ell.et Mart.)	33.6

Table 4. The disease rate of each block

		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Disease rate%
Block: 1	control	39	5	2	0	1	3	6.2
	inoculated	45	3	1	0	0	1	2.2
Block: 2	control	40	6	1	0	2	1	4.7
	inoculated	47	2	0	0	1	0	1.3

Table 5. The growth situation and antioxidant enzyme activities of eggplant seedling after 20 days

Treatment	Plant height (cm)	Dry weight (mg)	Chlorophyll Content (mg·L ⁻¹)	SOD (U·g ⁻¹)(FW)	CAT (U·g ⁻¹)(FW)	POD (U·g ⁻¹)(FW)
Control	7.912±0.169	4.717±0.173	0.536±0.134	86.210±0.210	21.075±0.208	35.883±0.100
Inoculated	12.390±0.103	6.300±0.009	0.751±0.125	175.150±0.195	47.906±0.106	65.122±0.251
Increasing rate (%)	56.61	33.55	40.1	103.2	127.3	81.5