

# Colonization of *Streptomyces felleus* YJ1 and Its Effects on Disease Resistant-Related Enzymes of Oilseed Rape

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## Abstract

*Streptomyces felleus* YJ1 has strong antagonism against *Sclerotinia sclerotiorum*, and it can be used for preventing this fungal disease in oilseed rape in the greenhouse. Under greenhouse conditions, we determined the colonization dynamic variation of YJ1 in soil and rape; and the changes of defensive enzymes of rape induced by YJ1 were measured. The results showed that, YJ1 could colonize chronically in soil; and it could also colonize in roots of rape and conduct to the stem and leaves, suggesting good colonization potential. After the rapes were processed by YJ1 fermentation liquid broth, the changes of defensive enzymes (SOD, PPO, POD etc.) in rape were measured. The results showed that, the activities of defensive enzymes changed significantly, especially in the four days later, the SOD, POD, PPO and PAL activities were significantly higher than that of inoculated with *S. sclerotiorum* and control, and maintained at a high level. Those results indicated that induced systemic resistance of rape maybe one of antagonistic mechanism of YJ1 against *S. sclerotiorum*.

**Keywords:** *Streptomyces felleus*, oilseed rape, colonization, disease resistant-related enzymes

## 1. Introduction

Sclerotinia stem rot, caused by the pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of the most serious diseases of oilseed rape (Ding et al., 2013). *S. sclerotiorum* is a kind of devastating worldwide pathogens and it can infect tomato, rapeseed, sunflower, soybean, broad beans, lettuce and many other economically important crops, besides its sclerotium that can survive for up to 8 years in soil has very strong resistance (Hegedus & Rimmer, 2005; Lamey, 2003; Bae & Knudsen, 2007). Oilseed rape is an important oil crop around the world, however the yield and quality of rape can be seriously affected by *S. sclerotiorum*, and the yield loss can be 10%~80% (Zhao & Meng, 2003; Ma et al., 2009). For the prevention of *S. sclerotiorum*, chemical control is the most commonly used strategy (Mueller et al., 2002). But using chemicals can cause environmental problems, and has negative influence on food safety, the health of human beings, etc (Demos & Korsten, 2006; Inbar, Menendez, & Chet, 1996). Therefore, many researchers have turned to studying using antagonistic microorganisms to prevent against *S. sclerotiorum*.

Presently, the research results have certificated that there are at least more than 30 kinds of fungi, bacteria or other microorganisms which have good control effects against *S. sclerotiorum* (Gao et al., 2010; Li et al., 2003; Chen et al., 2001; Jiang et al., 2000). Several species of *Bacillus* and *Pseudomonas* have been successfully used to control the fungus (Fernando et al., 2007); the mycoparasites *Trichoderma spp.* are the most used and studied, because they can effectively reduce the ascospores and sclerotia (Abdullah et al., 2008). They are widely used commercially and certified as available strains (Elad, 2000). In addition, actinomycetes (mainly *Streptomyces*) have also been reported for the control of *S. sclerotiorum*, because they can produce a variety of antibacterial substances and different types of lytic enzymes (chitinase, cellulase, endoglucanase etc.) that have inhibitory effects on many phytopathogens (Clardy et al., 2006; El-Tarabily et al., 1997). *Streptomyces spp.* 80, chitinase

producing *Streptomyces*, *Propionicimonas* sp. ENT-18, etc. were available to prevent *Sclerotinia sclerotiorum* (Fróes et al., 2012; Tahtamouni et al., 2006; Zucchi et al., 2010).

The colonization and adaptation of biocontrol bacteria in a certain environment, as well as good affinity ability in the target crops, is the key for that biocontrol strains to play the biocontrol effect effectively (Kloepper, 1992; Hu et al., 2009; Sturz et al., 1997). Inducing plant system resistance is one of the main antagonistic mechanisms of biocontrol bacteria. When plants are invaded by pathogens, their defense enzymes system [superoxide dismutase (SOD), peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) etc.] will be activated, and participate in various physiological metabolic processes, so that plant disease resistance responses are stimulated for preventing the invasion of pathogens (Kim et al., 2007). SOD, POD, PPO and PAL are closely related with plant disease resistance. SOD is endogenous active oxygen scavenger in plants and associated with lignin synthesis, and it can enhance plant resistance to pathogens (Dou et al., 2010); POD can catalyze the formation of lignin, thickening of plant cell wall to prevent bacteria invasion, meanwhile it also maintains the balance of active oxygen metabolism and protect the membrane structure in plants (Joseph et al., 1998); PAL is the key and rate limiting enzyme that participate in producing phytoalexin, lignans, phenolic compounds, and it was closely related with plants' system resistance (Wang & Zhu, 2002; Xue & Ouyang, 1992); Phenolic compounds can be oxidated by PPO into quinones materials that have inhibitory or killing effects on pathogens, and PPO is also involved in lignin formation (Wang et al., 2005; Volpin et al., 1995). *Bacillus subtilis* BY-2 can colonize in rape and be used for controlling *Sclerotinia sclerotiorum* effectively (Jiang et al., 2007). The *Streptomyces* spp.47W08, 47W10 etc., could be used as preventive biocontrol agents against *Phytophthora capsici*, and they could colonize in pepper roots while they were mixed with *P. capsici* in the inoculation; besides they could induce the improvement of PAL, PPO activities in the leaves of capsicum (Liang et al., 2005). Biocontrol agent XB16 which had a strong antagonism against *Fusarium oxysporum* colonized in banana plant commendably, and it could induce the activities of defense enzymes significantly increased (Sun et al., 2010).

The actinomycete strain YJ1 which was identified as *Streptomyces felleus*, was isolated from branches of Ginkgo and stored in our laboratory. It had significant effects by using this antagonistic actinomycetes to control *S. sclerotiorum* in laboratory, greenhouse and field trials (Yao, 2010). In this study, we tried to explore the colonization potential of *S. felleus* YJ1 and the changes of disease resistant-related enzymes activities in rape induced by YJ1, so that it has had a further theoretical basis for that *S. felleus* YJ1 could be used for biocontrol practice.

## 2 Materials and Methods

### 2.1 Strains, Culture Conditions and Medium

*Streptomyces felleus* YJ1 was provided by the plant pathology laboratory in Sichuan Agricultural University. *Sclerotinia sclerotiorum* was isolated from the sclerotia formed in the stem of diseased rape from Wenjiang, China.

*S. felleus* was maintained and preserved on Gause's medium No.1; Gause's liquid medium and millet liquid medium were used for cultivating bacteria liquid of YJ1. *S. sclerotiorum* was maintained on Potato Dextrose Agar (PDA). They were both stored at 4°C until required.

The medium compositions were as follows:

Gause's medium No.1 contained: soluble starch 20.0 g, KNO<sub>3</sub> 1.0 g, NaCl 0.5 g, K<sub>2</sub>HPO<sub>4</sub> 0.5 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01 g, agar 20 g, distilled water 1000 mL, pH 7.2.

PDA medium contained: potato 200 g, glucose 20 g, agar 18 ~ 20 g, distilled water 1000 mL.

Gause's liquid medium contained: the same as Gause's medium No.1 except agar.

Millet liquid medium: millet 10.0 g, glucose 10.0 g, peptone 3.0 g, NaCl 2.0 g, CaCO<sub>3</sub> 2.0 g, distilled water 1000 mL, pH 7.2-7.4.

### 2.2 YJ1 Colonization

#### 2.2.1 Streptomycin-Resistant Marker Strains

The strain YJ1 was incubated on Gause's medium No.1 which was added 20 µg/mL streptomycin, gradually with increasing streptomycin concentration until at the maximum concentration used (500 µg/mL). Every time the concentration was increased by 20 µg/mL. Then the mutation frequency, the physiological characteristics of the mutant strain and its inhibition effects against *S. sclerotiorum* were checked. Finally, the mutant strains were incubated in the flasks that contained Gause liquid medium added with 500 µg/mL streptomycin. The flasks were

put in the dark at 28°C on a rotary shaker at 180 r. min<sup>-1</sup> for 7 days. The bacteria concentration was adjusted to 2.5×10<sup>7</sup> cfu/mL. The bacterial suspension was stored at 4°C until required.

### 2.2.2 Determination of Colonization in Soil

The sterilized soil (the soil was collected from rhizosphere of oilseed rape) was arranged in the flowerpots (diameter 10 cm), every pot 1.5 kg soil, and then each pot was injected bacterial suspension of YJ1 10 mL, and all the pots were incubated at room temperature. Streptomycin-Resistant strains were separated from the soil every eight days, and then we made the determination of colonization. The experiments were repeated 3 times.

### 2.2.3 Determination of Colonization in Oilseed Rape

The oilseed rapes (Mianyou 16) were planted in the greenhouse. When the plants had 5~7 leaves, we inoculated YJ1 by applying a soil drench with 10 mL YJ1 suspension. Then we started sampling rape roots, stem and leaf tissues once per 15 days after one month and separated the Streptomycin-Resistant strains. Next, we determined the colony count of YJ1 and calculated per gram of bacteria content in the plant tissues.

## 2.3 Effect of YJ1 on Resistant-Related Enzyme Activity of Rape

### 2.3.1 Fermentation Broth Preparation

YJ1 was inoculated into millet liquid medium in 250 mL flasks. The flasks were incubated in the dark at 28°C on an eberbach rotary shaker at 160 r. min<sup>-1</sup> for 7 days to obtain the fermentation broth, and then adjusted bacteria concentration to 2.5×10<sup>7</sup> cfu/mL. The fermentation broth was stored at 4°C until required.

### 2.3.2 Inoculation and Sampling

The oilseed rapes (Mianyou 16) were planted in the greenhouse. When the plants had 7 leaves, the plants were treated respectively as follows: the first group, added YJ1 fermentation broth to the pots, every pot 30 mL; the second group, added distilled water 30 mL to each pot; the third group, added water 30 mL to each pot and inoculated with *S. sclerotiorum*. The experiments were repeated 3 times. After the oilseed rapes were treated for 0.5 hours, 2 days, 4 days, 6 days, 8 days, the rape seedling fresh leaves were gathered to measure enzymes activities.

### 2.3.3 Determination of Enzymes Activity

Took the leaves gathered in 2.3.2 back to the laboratory immediately, and respectively measured the SOD, POD, PPO, PAL enzymes activities. According to the methods of Li's, Wu's and Zhao's (Li, 2000; Wu, 2006; Zhao et al., 1998), the activities of SOD, POD, PPO and PAL were determined respectively.

## 3. Results

### 3.1 Streptomycin-Resistant Marker Strains

The strain YJ1 was incubated in Gause's medium No.1 which was added streptomycin, finally we got the mutant strain and its colony morphology was similar to the original strain, and its physiological characteristics and antagonistic effects against *S. sclerotiorum* remained unchanged nearly. Besides, when cultivated for 10 generations, the target strain was still resistant to 500 µg/mL streptomycin. Thus, it had the resistance marker trait.

### 3.2 Colonization of YJ1 in Soil

When *S. felleus* YJ1 was applied into the soil, the bacteria number had been increased, and the number rose slowly at the beginning, after 24 days increased rapidly, and the total number had reached 2.135×10<sup>9</sup> cfu/g in the 40th day (Figure 1). Next, the number of YJ1 did not change much, relatively stable. Thus, *S. felleus* YJ1 could colonize permanently in soil.

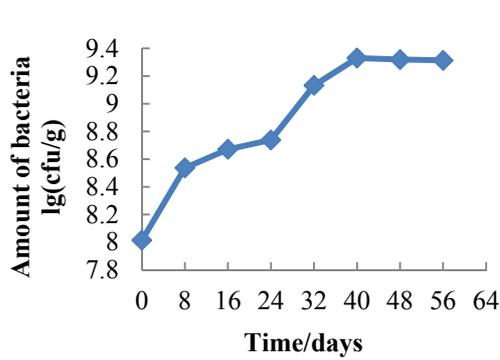


Figure 1. The colonization dynamics of biocontrol bacteria YJ1 in soil

lg(cfu/g) respects the common logarithm of the measured data

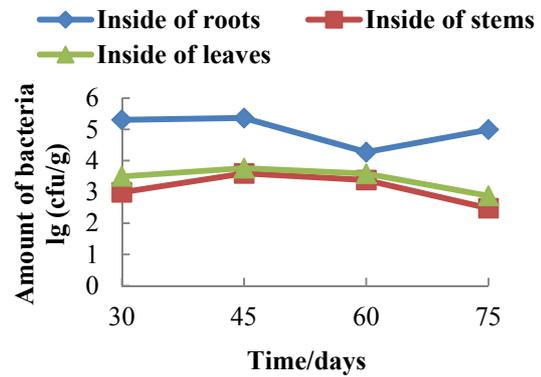


Figure 2. The colonization dynamics of biocontrol bacteria YJ1 in rapeseed

lg(cfu/g) respects the common logarithm of the measured data

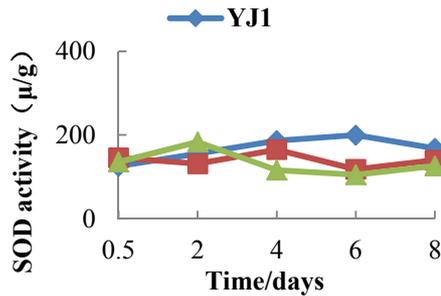


Figure 3. The change of SOD activity in leaves after fermentation liquid root irrigation and inoculated with *S. sclerotiorum*

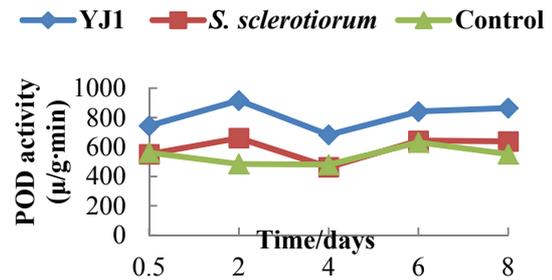


Figure 4. The change of POD activity in leaves after fermentation liquid root irrigation and inoculated with *S. sclerotiorum*

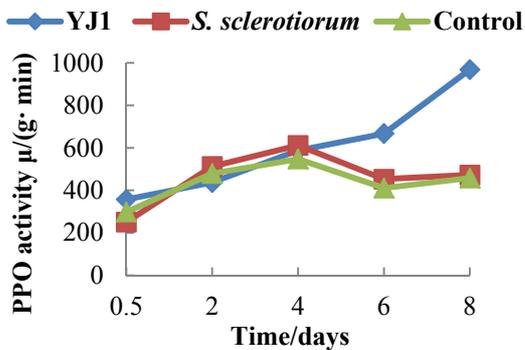


Figure 5. The change of PPO activity in leaves after fermentation liquid root irrigation and inoculated with *S. sclerotiorum*

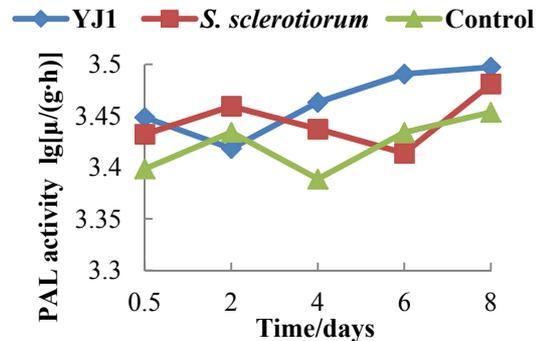


Figure 6. The change of PAL activity in leaves after fermentation liquid root irrigation and inoculated with *S. sclerotiorum*

lg[μ/(g·h)] respects the common logarithm of the measured data

Notes: Figure 3-6, YJ1 is treated by YJ1 fermentation broth; *S. sclerotiorum* is treated by *S. sclerotiorum*; and Control is treated by distilled water.

### 3.3 Colonization of YJ1 in Rape

Inoculated with YJ1 by using the root drench method, the colonization of *S. felleus* YJ1 in rape was determined. The test results showed that, YJ1 could colonize in rape roots, and could conduct from the roots to the stem and leaves for colonization; the colonization number: root > leaf > stem (Figure 2). After the rapes were treated by YJ1 for 30 days, the number of that YJ1 colonized in rape roots increased slightly firstly, decreased next, and then increased; the number in stems and in leaves increased firstly, and then decreased. In the 75th day after the treatments, the quantity of YJ1 colonized in roots was  $9.8 \times 10^4$  cfu/g; the stem and roots were  $3 \times 10^2$  cfu/g and  $7.5 \times 10^2$  cfu/g. Therefore, *S. felleus* YJ1 could colonize in rape for long time.

### 3.4 Effect of *S. felleus* YJ1 on Enzyme Activity of Rape

After treated by YJ1 fermentation liquid and inoculated with *S. sclerotiorum*, SOD, POD, PPO and PAL activities had significant changes. Treated by YJ1, SOD activity in rape leaves had a growth trend from the 0.5 day to 6th day, and to the eighth day began to decline (Figure 3). Besides, SOD activity was obviously higher than that inoculated with *S. sclerotiorum* and control after the rapes were treated for 4 days. Compared with the control, SOD activity of inoculated with *S. sclerotiorum* was higher except the second day (Figure 3).

After the oilseed rapes were treated by *S. felleus* YJ1, POD activity was significantly higher than control and that inoculated with *S. sclerotiorum* (Figure 4). POD activity had the trend of increasing at the beginning of 2 days; and decreased from the second day to the 4th, from the 4th day to the 8th it showed increasing trend. Inoculated with *S. sclerotiorum*, POD activity had no significant difference in the processing of the day comparing with control, and reached  $661.18 \mu\text{g}/(\text{g}\cdot\text{min})$  in the second day; and the next the POD activity changed similarly to control.

After treated by *S. felleus* YJ1, PPO activity increased continuously and reached  $968.38 \mu\text{g}/(\text{g}\cdot\text{min})$  in the eighth day. There had no obvious difference between control and YJ1 before the starting four days; and PPO activity was significantly higher than that inoculated with *S. sclerotiorum* and control from the fourth day to the eighth. After inoculated with *S. sclerotiorum*, the change trend of PPO activity was the same as control, but the enzyme activity was much higher after the rapes were treated for two days (Figure 5).

After treated by *S. felleus* YJ1, PAL activity in rape leaves had decreased from 0.5 day to 2 days; and the next, the enzyme activity increased continuously and was significantly higher than control after 2 days, and much higher than that inoculated with *S. sclerotiorum* after 4 days. Compared with control, PAL activity of leaves inoculated with *S. sclerotiorum* had the same tendency from 0.5 day to fourth and from the sixth day to eighth; besides, PAL activity was almost higher than control (Figure 6).

## 4. Discussion

As biocontrol strains, the important premise of that they play a prevention role is that whether they can colonize stably in the target plants or their surroundings (Lian et al., 2011). Therefore, many scholars have measured and analyzed the colonization potential of biocontrol strains, understand the characteristic of the strains, to make prevention effects of the strains reached the maximum and make the strains used reasonably (Baudoin et al., 2002; Hu et al., 2009). Combined with antibacterial activity determination, some researchers also treat colonization ability as an important index of strain screening to improve the success rate of strains screening (Guo et al., 1996). The researchers, such as Lian (2011), Long and Xiao (2003), Li et al. (2006), had reported that colonization of antagonistic bacteria in target crops and the results showed those strains could colonize in roots, stems and rhizosphere, and especially colonization in the roots was stable; in addition, the colonization ability of the strains in roots was stronger than that in stems, leaves; and colonization at the bottom of the stems was better than that of the upper stems. Through this study, we got the similar results: the strain YJ1 was colonized and transferred in rape tissues, and it could also proliferate in colonization sites. Besides, YJ1 could colonize in soil for long time. When biocontrol strains distribute steadily in plants and rhizosphere, it is easier for biocontrol strains to have the prevention effects (Andrews, 1992). In short, YJ1 was displayed good colonization in oilseed rape.

When the plants invaded by pathogens, the resistant-related enzymes (SOD, POD, PPO and PAL etc.) are activated and participate in many physiological processes, such as oxidation, lignification, response to pathogenic toxin, to prevent the invasion and reproduction of the pathogens. Therefore, SOD, POD, PPO and PAL are closely related with plant disease resistance. Some researchers, such as Yi et al. (2007) and Liu et al., (2010), have showed that SOD, POD, PPO, PAL activities were significantly enhanced when the target crops were treated by biocontrol strains. In this study, after inoculated with YJ1 fermentation broth by using the root drench method, SOD, POD, PPO, PAL activities showed obvious fluctuations, especially after the treatment for 4 days the enzymes activities were significantly higher than those inoculated with *S. sclerotiorum* and control, and maintained at a high level.

Those results indicated that induced systemic resistance of oilseed rape may be one of antagonistic mechanism of YJ1 against *S. sclerotiorum*.

Next, we can further study about antagonist material type of YJ1, development of agents and use methods etc, to ensure that this strain can be better used in biological control of *Sclerotinia sclerotiorum*.

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### References

- Abdullah, M. T., Ali, N. Y., & Suleman, P. (2008). Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*. *Crop Prot*, *27*, 1354-1359. <http://dx.doi.org/10.1016/j.cropro.2008.05.007>
- Andrews, J. H. (1992). Biological control in the phyllosphere. *Ann. Rev. Phytopathol*, *30*, 603-635. <http://dx.doi.org/10.1146/annurev.py.30.090192.003131>
- Bae, Y. S., & Knudsen, G. R. (2007). Effect of sclerotial distribution pattern of *Sclerotinia sclerotiorum* on biocontrol efficacy of *Trichoderma harzianum*. *Appl. Soil Ecol*, *35*, 21-24. <http://dx.doi.org/10.1016/j.apsoil.2006.05.014>
- Baudoin, E., Benizri, E., & Guckert, A. (2002). Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. *Applied Soil Ecology*, *19*(2), 135-45. [http://dx.doi.org/10.1016/S0929-1393\(01\)00185-8](http://dx.doi.org/10.1016/S0929-1393(01)00185-8)
- Chen, B. Y., Zhou, Y. C., & Lu, Z. P. (2001). Fermentation of *Trichoderma viride* and Suppression of Rapeseed *Sclerotinia* Stem Rot by the Fermentation Filtrate. *Chinese Journal of Biological Control*, *17*(2), 67-70.
- Clardy, J., Fischback, M. A., & Walsh, C. T. (2006). New antibiotic from bacterial natural products. *Nature Biotech*, *24*, 1541-1550. <http://dx.doi.org/10.1038/nbt1266>
- Demoz, B. T., & Korsten, L. (2006). *Bacillus subtilis* attachment, colonization, and survival on avocado flowers and its mode of action on stem-end rot pathogens. *Biological Control*, *37*, 68-74. <http://dx.doi.org/10.1016/j.biocontrol.2005.11.010>
- Ding, Y. J., Mei, J. Q., Li, Q. F., Liu, Y., Wan, H. F., Wang, L., ... Qian, W. (2013). Improvement of *Sclerotinia sclerotiorum* resistance in *Brassica napus* by using *B. oleracea*. *Genet Resour Crop Evol*, *60*, 1615-1619. <http://dx.doi.org/10.1007/s10722-013-9978-z>
- Dou, J. H., Yu, S. X., & Fan, S. L. (2010). SOD and Plant Stress Resistance. *Molecular Plant Breeding*, *8*(2), 359-364.
- Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Prot*, *19*, 709-714. [http://dx.doi.org/10.1016/S0261-2194\(00\)00094-6](http://dx.doi.org/10.1016/S0261-2194(00)00094-6)
- El-Tarabily, K. A., Hardy, G. E. S. J., Sivasithamparan, K., Hussein, A. M., & Kurtboke, D. I. (1997). The potential for the biological control of cavity-spot disease of carrots, caused by *Pythium coloratum*, by streptomycete and non-streptomycete actinomycetes. *New Phytol*, *137*, 495-507. <http://dx.doi.org/10.1046/j.1469-8137.1997.00856.x>
- Fernando, W. G. D, Nakkeeran, S., Zhang, Y., & Savchuk, S. (2007). Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. *Crop Prot*, *26*, 100-107. <http://dx.doi.org/10.1016/j.cropro.2006.04.007>
- Frões, A., Macrae, A., Rosa, J., Franco, M., Souza, R., Soares, R., & Coelho, R. (2012). Selection of a *Streptomyces* strain able to produce cell wall degrading enzymes and active against *Sclerotinia sclerotiorum*. *The Journal of Microbiology*, *50*(5), 798-806. <http://dx.doi.org/10.1007/s12275-012-2060-2>
- Gao, X. N., Chen, J. Y., & Huang, L. L. (2010). Screening of antagonistic endophytic bacteria and their roles in control of *Sclerotinia sclerotiorum*. *Chinese Journal of Pesticide Science*, *12*(2), 161-167.
- Guo, J. H., Wang, Y. J., & Li, J. (1996). Screen of biocontrol bacteria of plant wilt by inhibiting zones and root-colonization capacity. *Acta Phytopathologica Sinica*, *26*(1), 49-54.
- Hegedus, D. D., & Rimmer, S. R. (2005). *Sclerotinia sclerotiorum*: When “to be or not to be” a pathogen? *FEMS Microbiology Letters*, *251*, 177-184. <http://dx.doi.org/10.1016/j.femsle.2005.07.040>

- Hu, J. H., Zhang, F. J., & Lan, X. Q. (2009). Analysis of the colonization of tobacco rhizosphere bacterium swu31-2 and its control effect on tobacco bacterial wilt. *Plant Protection*, 35(5), 89-94.
- Inbar, J., Menendez, A. N. A., & Chet, I. (1996). Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. *Soil Biol. Biochem.*, 28, 757-763. [http://dx.doi.org/10.1016/0038-0717\(96\)00010-7](http://dx.doi.org/10.1016/0038-0717(96)00010-7)
- Jiang, D. H., Li, G. Q., Fu, & Y. P. (2000). Biocontrol of reinfection of oilseed rape stem rot caused by *Sclerotinia sclerotiorum* BY coniothyrium minitans and its survival on leaf of oilseed rape (*Brassica napus*). *Acta Phytopathologica Sinica*, 30(1), 60-65.
- Jiang, M. L., Zhao, R., Hu, X. J., Zhang, Y. B., & Wang, G. P. (2007). Colonization of antifungal endobacterium BY-2 in oilcrop rape and its control effect on disease caused by *Sclerotinia Sclerotiorum*. *Acta Phytopathologica Sinica*, 37(2), 192-196.
- Joseph, L. M., Tan, T. K., & Wong, S. M. (1998). Antifungal effects of hy2 drogen peroxide and peroxidase on spore germination and mycelia grow of *Pseudocercospora* species. *Canadian Journal of Botany*, 76(12), 2119-2124. <http://dx.doi.org/10.1139/b98-166>
- Kim, S. Y., Kim, J. Y., & Kim, S. H. (2007). Surfactin from *Bacillus subtilis* displays antiproliferative effect via apoptosis induction, cell cycle arrest and survival signaling suppression. *FEBS Letters*, 581(5), 865-871. <http://dx.doi.org/10.1016/j.febslet.2007.01.059>
- Klopper, J. W. (1992). A review of issues related to measuring colonization of plant roots by bacteria. *Canadian Journal of Microbiology*, 38(6), 667-672.
- Lamey, H. A. (2003). The status of *Sclerotinia sclerotiorum* on canola in North America. In *Proceedings of Sclerotinia initiative annual meeting*. MN, Bloomington.
- Li, G. Q., Huang, H. C., & Acharya, S. N. (2003). Antagonism and biocontrol potential of *Ulocladium stramon* *Sclerotinia sclerotiorum*. *Biological Control*, 28, 11-18. [http://dx.doi.org/10.1016/S1049-9644\(03\)00050-1](http://dx.doi.org/10.1016/S1049-9644(03)00050-1)
- Li, H. S. (2000). *Principle and technology of plant physiological and biochemical experiments*. Beijing: Science Press.
- Li, Q. Q., Luo, K., & Lin, W. (2006). Analysis on the colonization of entophytic bacteria B47 and its control on tomato bacterial wilt. *Acta Phytopathologica Sinica*, 33(4), 363-368.
- Lian, L. L., Xie, L. Y., & Chen, J. M. (2011). Colonization of biocontrol strain EN5 and its effects on rhizosphere soil microbial communities. *Plant Protection*, 37(2), 31-35.
- Liang, J. F., Xue, Q. H., Niu, X. L., & Li, Z. B. (2005). Root Colonization and Effects of Seven Strains of Actinomycetes on Leaf PAL and PPO Activities of Capsicum. *Acta Bot. Boreal. -Occident. Sin.*, 25(10), 2118-2123.
- Liu, Y. F., Li, M. R., Chen, Z. Y., Yu, J. J., Liu, Y. Z., Lie, Y. F., & Luo, C. P. (2010). The Inhibition Activities of Isolates YD4-6, NV11-4 and Their Induced Activities to Rice Defense Enzyme. *Microbiology China*, 37(12), 1753-1759.
- Long, L. K., & Xiao, C. G. (2003). Preliminary study on the colonization of endophytic bacterium 01-144 in tomato root and srem. *Microbiology*, 30(5), 53-56.
- Ma, H. X., Feng, X. J., Chen, Y., Chen, C. J., & Zhou, M. G. (2009). Occurrence and characterization of dimethachlon insensitivity in *Sclerotinia sclerotiorum* in Jinagsu Province of China. *Plant Dis.*, 93(1), 36-42. <http://dx.doi.org/10.1094/PDIS-93-1-0036>
- Mueller, D. S., Dorrance, A. E., & Derksen, R. C. (2002). Efficacy of fungicides on *Sclerotinia sclerotiorum* and their potential for control of sclerotinia stem rot on soybean. *Plant Dis.*, 86, 26-31. <http://dx.doi.org/10.1094/PDIS.2002.86.1.26>
- Sturz, A. V., Christie, B. R., & Matheson, B. G. (1997). Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol Fertil Soils*, 25, 13-19. <http://dx.doi.org/10.1007/s003740050273>
- Sun, J. B., Wang, Y. G., Zhao, P. J., Sun, H. Y., & Peng, M. (2010). Colonization of Biocontrol Strain XB16 against *Fusarium* Wilt Pathogen of Banana and Its Effect on Defense-related enzymes. *Chinese Journal of Tropical Crops*, 31(2), 212-216.

- Tahtamouni, M. E. W., Hammed, K. M., & Saadoun, I. M. (2006). Biological control of *Sclerotinia sclerotiorum* using indigenous chitinolytic actinomycete in Jordan. *Plant Pathol J*, 22, 107-114. <http://dx.doi.org/10.5423/PPJ.2006.22.2.107>
- Volpin, H., Phillips, D. A., & Okon, Y. (1995). Suppression of an Isoflavonoid Phytoalexin Defense Response in Mycorrhizal Alfalfa Roots. *Plant Physiology*, 108(4), 1449-1454.
- Wang, M. L., Hu, Z. L., & Zhou, M. Q. (2005). Advances in Research of Polyphenol Oxidase in plants. *Chinese Bulletin of Botany*, 22(2), 215-222.
- Wang, S. R., & Zhu, K. G. (2002). Advances of research on systemic acquired resistance in plants. *Chinese Journal of Eco-Agriculture*, 10(2), 32-35.
- Wu, J. (2006). *The Spatial Distribution of Aculops lycopersici and the Physiological and Biochemical Response of Tomato to Damage*. Dissertation, Yangzhou University.
- Xue, Y. L., & Ouyang, G. C. (1992). Metabolic basis of plant disease resistance. *Plant physiology and molecular biology*. Beijing: Science Press.
- Yao, J. (2010). *Study on the Biocontrol of Sclerotinia sclerotiorum by Antagonistic Actinomycetes*. Dissertation, Sichuan Agricultural University.
- Yi, L., Xiao, C. G., & Ma, G. H. (2007). Antagonistic Endophytic Epiphytic Bacteria, and Their Combination, Induced Tobacco to Resist against Brown Spot Disease. *Chinese Journal of Biological Control*, 23(2), 165-169.
- Zhao, J. W., Xiang, L., & He, F. X. (1998). Relationship between some enzyme activity and resistance to *Sclerotinia sclerotiorum* of new strain selected by intergeneric hybridization in Brassica napus. *Chinese Journal of Oil Crop Sciences*, 20(1), 38-41. <http://dx.doi.org/10.1046/j.1439-0523.2003.00784.x>
- Zhao, J., & Meng, J. (2003). Detection of loci controlling seed glucosinolate content and their association with *Sclerotinia* resistance in *Brassica napus*. *Plant Breed*, 122(1), 19-23.
- Zucchi, T. D., Almeida, L. G., Dossi, F. C. A., & Fernando, L. C. (2010). Secondary metabolites produced by *Propionicimnas sp.* (ENT-18) induce histological abnormalities in the sclerotia of *Sclerotinia sclerotiorum*. *Biocontrol*, 55(6), 811-819. <http://dx.doi.org/10.1007/s10526-010-9295-9>

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