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Full Length Research Paper

Antibiotic activity of bacterial isolates associated with entomopathogenic nematodes

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In the present study, we isolated 23 strains of symbiotic bacteria from 23 entomopathogenic nematodes strains gathered in different vegetation from different regions of China. The antibiotic activities of all these bacteria strains isolated were evaluated in the laboratory. All the bacteria effectively inhibited seven kinds of plant pathogenic fungi (*Bipolaris sorokinianum*, *Fusarium graminearum*, *Fusarium moniliforme*, *Cordana musae* (*zimm.*) Hohn, *Colletotrichum gloeosporiodies*, *Alternaria solani* and *Alternaria alternata* (*Fries*) Keissler) cultured on agar plates. Among these strains, strain SY5 was the most effective symbiotic bacteria strain, which was further selected as the highly virulent bacteria for the adversity resistance study. The adversity resistance showed that the stability of the antibiotic activity against different plant pathogenic fungi was different. The antibiotic activity against *A. solani* was the most stable and the inhibiting rate was not affected by treatment in a 50°C water bath for 60 min and in 100°C for 10 min, ultraviolet light exposure for 120 min and storing at room temperature for 90 days.

Key words: Entomopathogenic nematode symbiotic bacteria, Inhabiting rate, temperature, ultraviolet light exposure and duration of maintenance.

INTRODUCTION

Entomopathogenic nematode (EPN) and theirs symbiotic bacterium (EPB) are worldwide used as microbial control agents and have been isolated from many islands and all inhabited continents (Lee et al., 2002). EPN (genera *Steinernema* and *Heterorhabditis*) kill insects with the aid of a mutualistic symbiosis with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for Steinernematidae and Heterorhabditidae, respectively) (Ffrench-Constant and Bowen, 2000). *Xenorhabdus* and *Photorhabdus* are Gram-negative bacterium, belonging to Enterobacteriaceae and released from the nematodes into the host haemolymph within 5 h of invasion, and the larvae are generally killed within 48 h (Shapiro-Ilan et al., 2008). The secondary metabolites produced by the EPB overcome the insect immune system, kill the insect and inhibit the growth of various fungal and bacterial competitors (Chen et al., 1996). By so doing, the bacterial symbionts are believed to prevent putrefaction of the insect cadaver and establish conditions that favor the development of both the nematode and bacterial symbionts (Wang and Zhang, 2007).

Xenorhabdus and *Photorhabdus* secrete a wide variety of substance into the culture medium including toxins, lipases, proteases, antibiotics and lipopolysaccharides (Caldas et al., 2002; Richards et al., 2008; Hu and Webster, 2000). Dutky et al. (1964) suggested that the bacteria which live as symbionts of the entomopathogenic nematode *Steinernema carpocapsae* produce antibiotics which were confirmed by Akhurst (1982) who demonstrated the antibiotic activity of cultures of *Xenorhabdus* spp. against a wide variety of micro-organisms, including fungi, Gram-positive *Micrococcus*, *Staphylococcus* and *Bacillus*, as well as Gram-negative

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bacteria. The early and continued presence of antibiotics and their commonality across the different nematode-bacterium-insect interactions appears to have at least two functions. The antibiotics help to minimize competition from non-symbiotic bacteria, especially early in the infection, and subsequently prevent microbial putrefaction of the nematode-infected insect cadavers. Nevertheless, there is substantial variation in the type of antibiotic metabolite produced by different species of bacterial symbiont. Several bioactive secondary metabolites have been reported from cultures of EPB (Sundar and Chang, 1993), such as xenomins (Webster et al., 2002), xenorxids (Li et al., 1998), xenorhabdins (McInerney et al., 1991a), xenocoumacins (McInerney et al., 1991b) and nematophin (Li et al., 1997). These compounds were reported as showing in vitro activity against Gram-positive bacteria and fungi (Furgani et al., 2008).

The discovery and successful application of new natural antibiotics, such as those produced by entomopathogenic nematodes and their symbiont bacteria, may provide an alternative way of plant disease control. Because of the plant diseases, 12% of the agricultural products will be loss every year (Wang and Chen, 2000). Therefore, we decided to search for the effective symbiont bacteria with potential for use in the plant disease control. In the present study, seven kinds of plant pathogenic fungi (*Bipolaris sorokinianum*, *Fusarium graminearum*, *Fusarium moniliforme*, *Cordana musae* (*zimm.*) Hohn, *Colletotrichum gloeosporiodies*, *Alternaria solani* and *Alternaria alternata* (*Fries*) Keissler) which caused the wheat, rice, vegetable and fruit diseases were used as the object for evaluating the antibiotic activities of the symbiotic bacteria in the laboratory. This study is the first step towards achieving those goals.

MATERIALS AND METHODS

Isolation of the Entomopathogenic nematodes and bacterial symbionts

The entomopathogenic nematodes trapped from the soil samples by fifth instar larvae of *Galleria mellonella* which were obtained from Pest Biological Control Laboratory, Shenyang Agricultural University. The fifth instar larvae of *Galleria mellonella* were put into the jar which was filled with the soil samples. After a week, the nematodes were gathered and propagated from the dead larvae, and stored at 8°C.

Twenty-three strains of entomopathogenic nematodes were isolated from 359 soil samples which was collected from different regions of China. All of the entomopathogenic nematodes used in this study are listed in Table 1. The names of the entomopathogenic nematodes were given according to soil sample coded in our lab. Five reference nematode strains were used as the control in the study. *S. carpocapsae* A24 was obtained from Guangdong Entomological Institute, China. *Steinernema glaseri* NC34 was obtained from the State Key Laboratory for Biocontrol, Zhongshan University, China. *Steinernematidae feltiae* Otio, *Heterorhabditidae zealandica* (HZ) and *Heterorhabditidae bacteriphora* 15-2 were kindly provided by Plant Protection Institute, the Chinese Academy of Agricultural Sciences, China.

Isolation of the bacterial symbionts

Cadavers of greater wax moth larvae, *G. mellonella*, infected with different strains entomopathogenic nematodes were surface sterilized in 70% alcohol for 2 min, transferred to clean tissue to dry in a laminar airflow cabinet for 3 min, opened with sterile needles and a drop of haemolymph was streaked on to the differentiation medium (NBTA medium containing 45 g nutrient agar, 25 mg bromothymol blue and 40 mg triphenyl tetrazolium in 1 L distilled water) in 9 cm Petri-dishes and incubated at 28°C. The phase status (phase I colonies are blue, while phase II colonies are red) was selected and purified on the NBTA. After 48 h single colonies of the bacteria from cadavers infected with entomopathogenic nematode were inoculated on nutrient agar plates and sub-cultured continually until colonies of uniform size and morphology were obtained. The pathogenicity of isolates was confirmed by injecting cells of the bacteria into *G. mellonella* larvae.

Twenty-eight strains of symbiotic bacteria were isolated from 5 species entomopathogenic nematodes saved in the laboratory and 23 species in the soil samples collected from different regions of China. The bacterial strain names were given according to the entomopathogenic nematode hosts coded in our lab.

Production of bacterial cell

A colony was inoculated into nutrient broth (18 g nutrient broth in 500 ml distilled water) in a flask and placed in a shaking incubator at 160 revolution /min for 40 h at 27°C. The concentration of bacterial cells in the broth suspension was determined on a spectrophotometer at 600 nm wavelength.

Inhibition of fungal growth by the bacterial symbionts

Seven kinds of plant pathogenic fungi (*B. sorokinianum*, *F. graminearum*, *F. moniliforme*, *C. musae* (*zimm.*) Hohn, *C. gloeosporiodies*, *A. solani* and *A. alternata* (*Fries*) Keissler) were kindly provided by Shenyang Chemical Engineering Research College. This bioassay is based on measuring the diameter of the inactivation circle. 1 ml suspension of the bacterial symbionts (2.7×10^6 cells/ml) was added to 25 ml of cooling PDA agar in 9 cm Petri dishes, control dishes had nutrient broth only. When solid, a 5 mm of the plant pathogenic fungi plug was added to the centre of the dish. All dishes were sealed and incubated at 28°C. Three batches were repeated. Observations of the inhibition were recorded after 5 days. The diameter of contrast and the treatment were measured by the cross method. The inhibiting rate of plant pathogenic fungi was calculated by the formula (The inhibiting rate against plant pathogenic fungi = 100 × (the diameter of contrast–the diameter of treatments)/contrast diameter).

The adversity resistance of the bacterial symbionts

After cultivation, the broth of the highly antibiotics activity bacteria was put in a water bath set at 50°C temperature for different time (5, 10, 30 and 60 min) and 100°C for 10 min as the test samples. Each temperature was given the same treatment nutrient broth as the negative control and the broth which was not treated by the temperature as the positive control. The method of bioassay was same as the above. The inhibiting rate to different plant pathogenic fungi were measured and calculated.

The above experiment was repeated with the resistant of ultraviolet light exposure and the duration of maintenance at room temperature. The broth was exposed under the 18 W ultraviolet light 60 cm for 10, 20, 30, 60 and 120 min as the test samples for the

Table 1. The inhibiting rate of symbiotic bacteria strains against seven kinds of plant pathogenic fungi (means ± SE %).

Nematode strain ID	Source sampled	Vegetation	*B. sorokinianum*	*F. graminearum*	*F. moniliforme*	*C. musae* (*zimm.*) Hohn	*C. gloeosporiodies*	*A. solani*	*A. alternata* (*Fries*) Keissler
Reference strain									
S. carpocapsae A24	America	Unknow	24.23±3.79 hij	43.95±4.36 cde	21.21±0.58 l	54.55±2.65 abcde	75.77±3.06 abc	66.68±2.52 abcdef	25.77±0.58 d
S.glaseri NC34	America	Unknow	37.86±3.00 cdef	50.00±3.46 bcd	36.36±1.00 ghijkl	37.86±3.21 defg	68.18±0.00 bcd	48.5±2.08 cdefghi	57.59±1.53 a
S. feltiae Otio	Beijing, China	Unknow	28.77±2.52 fghij	59.09±1.53 bcd	33.33±1.53 hijkl	42.41±4.93 bcdefg	65.14±3.21 bcde	37.86±0.58 hi	54.55±2.00 ab
H.zealandica (HZ)	New Zealand	Unknow	25.73±1.00 ghij	62.14±1.73 bc	25.76±0.58 kl	18.18±1.00 g	65.14±3.51 bcde	59.09±4.00 abcdefghi	43.95±2.52 abc
H.bacteriphora 15-2	Neimenggu, China	Unknow	19.68±2.00 jk	59.09±1.73 bcd	56.06±2.08 cde	53.05±1.53 abcdef	62.14±1.15 bcde	65.14±4.93 abcdefg	56.05±0.58 a
Strain sampled									
Ht1	Shanghai, China	Upland field	13.64±3.06 k	57.59±2.00 bcd	28.79±5.03 jkl	21.23±1.53 fg	53.05±2.31 def	74.23±4.62 abc	50.00±1.00 ab
1	Tieling, Liaoning, China	Hemp	24.23±5.13 hij	60.59±3.06 bcd	54.55±2.65 cdef	45.45±4.00 bcdefg	65.14±0.58 bcde	71.23±1.15 abcd	54.55±2.00 ab
2	Henan, China	Wheat	25.73±3.61 ghij	63.64±2.00 bc	24.24±0.58 kl	46.95±2.31 abcdefg	42.41±1.53 f	33.32±3.21 i	28.77±1.53 cd
SY5	Shenyang,Liaoning, China	Persimmon	56.05±1.15 a	82.41±1.15 a	65.15±3.20 abc	61.36±2.29 abcde	87.86±2.50 a	60.59±5.35 abcdefgh	50.77±3.19 ab
8	Gansu, China	Wheat	30.27±5.00 efghi	74.23±1/04 ab	51.52±3.21 cdefg	37.86±3.21 defg	63.64±1.00 bcde	40.91±2.65 fghi	48.50±2.08 ab
03100C	Shandong, China	Tomato	30.27±3.06 efghi	53.05±2.65 bcd	59.09±1.00 bcd	36.36±6.08 defg	60.59±2.89 bcdef	45.45±1.00 defghi	43.95±1.53 abc
03101Y	Shanxi, China	Corn	25.73±2.65 ghij	57.59±0.42 bcd	60.61±1.53 abcd	21.23±1.53 fg	59.09±0.00 cdef	54.55±1.00 bcdefghi	45.45±2.65 ab
03121H	Tianjin, China	Cucumber	34.82±4.73 defg	66.68±2.02 abc	25.76±2.08 kl	50.00±4.00 abcdefg	75.77±1.15 abc	54.55±1.73 bcdefghi	51.50±1.53 ab
0312-4	Neimenggu, China	Potato	21.18±1.73 ijk	39.41±0.58 de	75.76±1.53 a	51.50±1.53 abcdef	65.14±2.08 bcde	68.18±2.65 abcde	50.00±1.00 ab
0321-2	Gansu, China	Wheat	21.18±1.15 ijk	57.59±1.04 bcd	31.82±1.73 ijkl	65.14±4.51 abcde	57.59±1.15 cdef	83.32±2.88 a	53.05±1.53 ab
0355BC	Neimenggu, China	Chinese cabbage	24.23±4.16 hij	36.36±2.19 de	37.88±1.53 fghijkl	33.32±5.13 efg	69.68±1.15 bcd	53.05±1.53 bcdefghi	56.05±2.52 a
0355DJ	Neimenggu, China	Kidney beans	34.82±1.73 defg	42.41±2.95 cde	30.30±1.53 ijkl	39.41±4.04 cdefg	65.14±0.58 bcde	42.41±2.52efghi	59.09±2.65 a
0356H	Liaoning, China	Ash	13.64±2.52 k	63.64±2.65 bc	72.73±1.00 ab	68.18±4.36 abcd	63.64±2.65 bcde	40.91±2.65 fghi	43.95±2.08 abc
0361D	Shenyang, Liaoning, China	Sweet potato	33.32±1.53 defgh	57.59±1.73 bcd	28.79±0.58 jkl	68.18±2.00 abcd	53.05±1.53 def	54.55±1.00 bcdefghi	56.05±1.53 a
0362W	Tianjin, China	Asparagus	22.73±1.00 ijk	42.41±2.31 cde	39.39±0.58 efghijk	48.05±4.04 abcdefg	65.14±0.58 bcde	39.41±4.04 ghi	56.05±2.08 a
0384q	Liaoyang, Liaoning, China	Green pepper	25.73±2.08 ghij	72.73±1.53 abc	46.97±2.08 defghi	74.23±2.08 ab	78.77±2.31 ab	65.14±0.58 abcdefg	53.05±1.53 ab
0385D	Benxi, Liaoning, China	Kidney beans	21.18±1.15 ijk	59.09±3.79 bcd	34.85±1.15 ghijkl	78.77±1.53 a	60.59±0.58 bcdef	68.18±3.61 abcde	48.50±1.53 ab
0386L	Shenyang, Liaoning, China	Soybean	24.23±2.08 hij	66.68±3.46 abc	59.09±1.00 bcd	71.23±1.53 abc	62.14±2.89 bcde	65.14±1.53 abcdefg	43.95±1.53 abc
0389	Shenyang, Liaoning, China	Willow	40.91± 3.00 cd	57.59±1.00 bcd	60.61±0.58 abcd	65.14±0.58 abcde	56.05±4.93 def	63.64±4.58 abcdefgh	54.55±2.00 ab
0396Y	Shenyang, Liaoning, China	Cherry	21.18±1.53 ijk	54.55±2.52 bcd	50.00±2.00 cdefgh	75.55±1.53 ab	59.09±1.00 cdef	68.18±2.00 abcde	37.86±1.53 bcd
0397C_2	Benxi, Liaoning, China	Chinese cabbage	39.36±4.51 cde	34.86±2.08 e	51.52±2.31 cdefg	57.59±5.86 abcde	46.95±2.31 ef	66.68±1.15 abcdef	46.95±1.53 ab
0398B	Shenyang, Liaoning, China	Chinese cabbage	45.45±1.53 bc	62.14±1.15 bc	45.45±1.00 defghij	45.45±4.36 bcdefg	54.55±1.00 def	68.18±1.00 abcde	45.45±1.00 ab
0399H	Shenyang, Liaoning, China	Soybean	50.00±2.89 ab	59.09±2.31 bcd	50.00±1.00 cdefgh	71.23±3.06 abc	60.59±3.06 bcdef	75.77±1.53 ab	54.55±2.00 ab

resistant of ultraviolet light exposure experiment. Each time have the same treatment nutrient broth as the negative control and the broth which was not treated by the ultraviolet light as the positive control. The broth was stored at room temperature for 1, 2, 3, 7, 30, 60, 90 and 150d as the test samples for evaluating the duration of maintenance. Each time have the same treatment nutrient broth as the control.

Statistical analysis

The data were analyzed by one-way ANOVA, SPSS 12.0 software. Repeated measures ANOVA was used to analyze

the data on the inhibiting rate and differences between treatments were determined using contrasts. Duncan was used to test the differences between treatments. All comparisons were considered significant at $p<0.05$.

RESULTS

Inhibition of fungal growth by the symbiotic bacteria

Twenty-eight strains of symbiotic bacteria were isolated from 5 species of entomopathogenic nematodes saved in the laboratory and 23 species in the soil samples collected from different regions of China. The antibiotic activities of 28 strains of symbiotic bacteria were evaluated in the laboratory by bioassays.

Antibiotic production by *Xenorhabdus* and *Photorhabdus* differs qualitatively and quantitatively in the strain types and species (Fang et al., 2010), and the phase II cells are low or lacking in antibiotic activity (Boemare et al., 1988). So, *Xenorhabdus nematophila*, *Xenorhabdus poinarrii*, *Xenorhabdus bovienii*, *Photorhabdus temperate* and *P. luminescens* were used as reference strains and the phase I cells are used in this study. This 5 reference strains were the symbiont of *S. carpocapsae* A24, *S. glaseri* NC34, *S. feltiae* Otio, *H. zealandica* (HZ) and *H. bacteriphora* 15-2, respectively (Tailliez et al., 2006; Boemare, 2002), which had highly insecticidal activity to pests (Han et al., 2008; Jagdale et al., 2005; Qiu et al., 2005; Sun et al., 2006; Bussaman et al., 2006).

The antibiotic activity was assessed qualitatively on the basis of the size of the inhibition zone. According to the bioassay results, all bacteria inhibited the growth of the pathogen and the antibiotic activities of the different reference species were different against the same and different plant pathogenic fungi (Table 1).

As shown in Table 1, all bacteria used had significant effect on the seven kinds of plant pathogenic fungi. The most effective strain was the symbiotic bacteria strain SY5. The broth of stain SY5 had the highest antibiotic activity against *B. sorokinianum*, *F. graminearum* and *C. gloeosporiodies* (the inhibiting rate of plant pathogenic fungi were 56.05 ± 1.15, 82.41 ± 1.15 and 87.86 ± 2.50, respectively). But to *F. moniliforme*, *C. musae* (*zimm.*) Hohn, *A. solani* and *A. alternata* (*Fries*) Keissler, the broth of strain 0312-4, 0385D, 0321-2 and 0355DJ showed the highest antibiotic activity respectively. The inhibiting rates were 75.76 ± 1.53, 78.77 ± 1.53, 83.32 ± 2.88, 59.09 ± 2.65, respectively and were not significantly different from strain SY5 (65.15 ± 3.20, 61.36 ± 2.29, 60.59 ± 5.35 and 50.77 ± 3.19) by SPSS analysis. But the inhibiting rates of the strain 0312-4, 0385D, 0321-2 and 0355DJ against *B. sorokinianum*, *F. graminearum* and *C. gloeosporiodies* were much lower than that of strain SY5.

Taken together, the broth of strain SY5 had highest antibiotic activity against *B. sorokinianum*, *F. graminearum*, *F. moniliforme*, *C. musae* (*zimm.*) Hohn, *C. gloeosporiodies*, *A. solani* and *A. alternata* (*Fries*) Keissler. Therefore, the symbiotic bacteria strain SY5 was selected as the highly virulent symbiotic bacteria for further study.

Effects of temperature on antibiotic activity

Temperature influence of antibiotic activity was examined for understanding the stability and persistence of the symbiotic bacteria strain SY5 to different plant pathogenic fungi. The results showed that the activity of the total antibiotics declined with increasing temperature and the extending time (Table 2). To different plant pathogenic fungi, the stability of antibiotics was different. To *B. sorokinianum* and *F. moniliforme*, the inhibiting rates were no significant difference between the control and the 50°C treatment in different time, but the antibiotic activities declined significantly after in a 100°C water bath for 10 min. There have some fluctuations on the inhibiting rates against *C. musae* (*zimm.*) Hohn and *C. gloeosporiodies*. Except the treatment of 50°C for 10 min to *C. musae* (*zimm.*) Hohn and 50°C for 60 min to *C. gloeosporiodies*, the inhibiting rates were not significantly different between the control and the other treatments.

The antibiotic activities against *A. solani* and *A. alternate* (*Fries*) Keissler were the most stable because the inhibiting rates were no statistical difference between the control and all the temperature treatment. The most unstable treatment was the antibiotic activity against *F. graminearum*, the inhibiting rates declined quickly after in a 50°C water bath for 30 min, 60 min and 100°C for 10 min.

Effects of ultraviolet light exposure on antibiotic activity

The effects of ultraviolet ray on antibiotic activity by the symbiotic bacteria strain SY5 was examined (Table 3). The stability of antibiotics was different among the different plant pathogenic fungi. The most stable treatments were the inhibition of *F. moniliforme*, *C. musae* (*zimm.*) Hohn, *A. solani* and *A. alternate* (*Fries*) Keissler; the inhibiting rates were not significantly different between the control and all the treatment in different time. The antibiotic activity to *F. graminearum* was the most unstable because the inhibiting rates declined quickly after exposure to the ultraviolet light for 20 min.

The inhibiting rate against *B. sorokinianum* has no statistical difference between the control and the treatments except the treatment of ultraviolet light exposure for 120 min. To *C. gloeosporiodies*, the inhibiting rate was fluctuant. There was no significant different between the control and the treatments of ultraviolet light exposure for 10, 60 and 120 min, but the antibiotic activities were declined significantly after exposure to the ultraviolet light for 20 and 30 min.

Table2. Inhibiting rate of strain SY5 broth in different temperature (means ± SE %).

Plant pathogenic fungi	Room temperature	50°C				100°C
		5 min	10 min	30 min	60 min	10 min
Bipolaris sorokinianum	55.32±1.45 a	49.23±0.33 ab	52.95±1.86 a	48.50±2.33 ab	50.32±1.15 ab	39.41±1.52 b
Fusarium graminearum	81.82±1.20 a	77.23±2.85 ab	71.23±2.19 abc	45.45±2.08 bc	40.91±0.67 c	36.36±2.62 c
Fusarium moniliforme	66.24±2.31 a	60.64±2.67 a	51.55±1.53 ab	55.68±0.88 ab	45.68±0.58 ab	33.36±1.34 b
C. musae (*zimm.*) Hohn	65.14±1.86 ab	72.73±0.67 a	56.05±0.58 b	77.27±2.65 a	68.18±0.88 ab	69.68±3.03 ab
Colletotrichum gloeosporiodies	81.82±4.55 a	75.76±2.62 ab	72.73±4.55 ab	75.76±4.01 ab	69.70±3.03 b	72.73±4.55 ab
Alternaria solani	57.57±2.62 a	53.03±2.62 a	53.03±6.94 a	50.76±4.73 a	52.27±2.27 a	50.00±4.55 a
Alternaria alternata (*Fries*) Keissler	57.27±2.41 a	42.42±5.46 a	50.00±6.18 a	53.03±4.01 a	51.52±4.01 a	51.52±2.60 a

Table 3. Inhibiting rate of strain SY5 broth in ultraviolet light exposure (means ± SE %).

Plant pathogenic fungi	No ultraviolet light	In ultraviolet ray				
		10 min	20 min	30 min	60 min	120 min
Bipolaris sorokinianum	55.32±1.76 a	49.23±1.33 ab	52.95±1.67 a	48.50±0.67 ab	50.32±1.33 ab	39.41±1.02 b
Fusarium graminearum	81.82±1.86 a	77.23±0.88 ab	71.23±0.67 abc	45.45±0.33 bc	40.91±2.85 c	36.36±0.67 c
Fusarium moniliforme	66.24±2.02 a	66.64±1.02 a	59.09±1.20 a	57.55±1.53 a	65.18±1.76 a	57.55±0.33 a
Cordana musae (*zimm.*) Hohn	65.14±0.67 a	65.14±0.58 a	60.59±0.67 a	65.14±0.88 a.	56.05±1.53 a	62.09±1.00 a
Colletotrichum gloeosporiodies	81.82±4.55 ab	77.27±4.55 ab	68.18±4.46 b	68.18±4.55 b	84.85±2.62 a	81.82±4.55 ab
Alternaria solani	57.57±2.62 a	65.15 ±6.06 a	63.64±4.55 a	59.09±4.55 a	63.64±0.00 a	66.67± 6.60 a
Alternaria alternata (Fries) Keissler	57.27±2.41 a	51.52±4.01 a	48.79±2.92 a	53.03±5.46 a	56.06±2.62 a	58.27±3.36 a

Effects of the maintenance duration on antibiotic activity

The broth of the symbiotic bacteria strain SY5 was stored at room temperature for 5 months for evaluating the duration of maintenance (Figure 1). Differences among the duration of maintenance were produced with the different plant pathogenic fungi. Generally, after storing for 5 months, the broth showed less antibiotic activity than the fresh broth. In all samples, antibiotic activity against *C. gloeosporiodies* was the most obvious changed with the extension of the storage time. After storing at room temperature for 3 days, the inhibiting rate decreased significantly. However, the antibiotic against *A. solani* was the most stable, and did not change even when stored at room temperature for 1 to 90 days. The treatments to *B. sorokinianum* and *F. graminearum* were established antibiotic activity (based on the inhibiting rate) to be stable for up to 60 days. The inhibiting rate against *B. sorokinianum* declined thereafter, but for *F. graminearum*, the inhibiting rate declined by 90 days and then increased by 150 days. The antibiotic activities against *C. musae* (*zimm.*) Hohn and *A. alternata* (*Fries*) Keissler exhibited a continuous decline from 7 days, and the inhibiting rate against *F. moniliforme* declined after storing for 7 days.

DISCUSSION

It has been widely accepted the *Xenorhabdus* and *Photorhabdus* inhibit the growth of other microorganisms in insects infected with

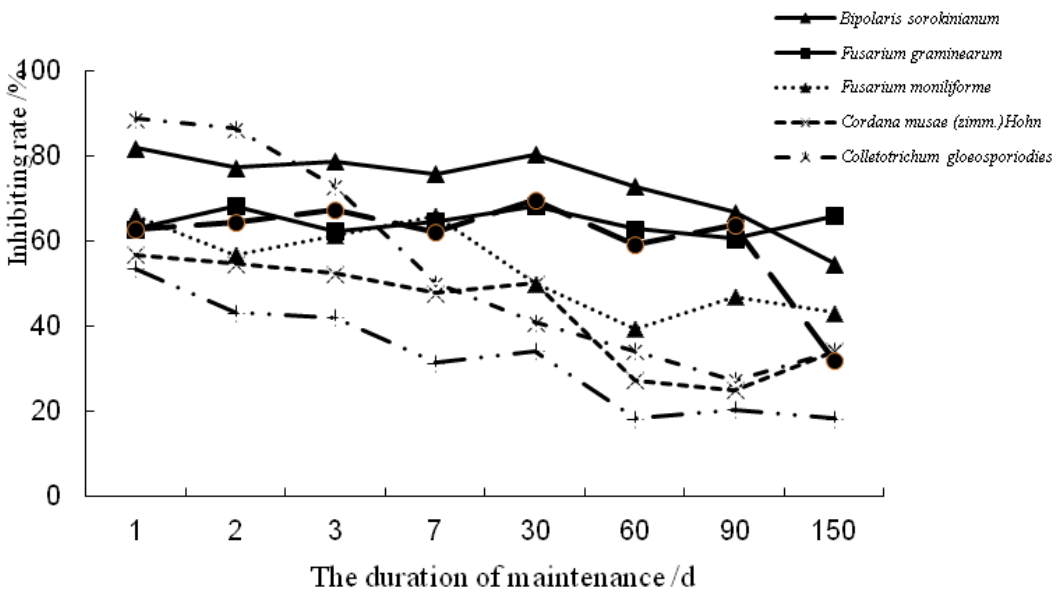

Figure 1. Effects of the maintenance duration on antibiotic activity by stain SY5.

Steinernematidae and Heterorhabditidae. Antibiotic production is now known to be a very common characteristic of *Xenorhabdus* and *Photorhabdus* species (Paul et al., 1981), and the compounds produced as antibiotics are quite diverse (Richardson et al., 1988; Sztaricskai et al., 1992). So the antibiotic activities of the different species were different to the same and different plant pathogenic fungi. The result of this study are consistent with previous. The antibiotics of all the strains used in this study were powerful in either bioassay, but the sensitivity of the isolates differed from each other to the same or the different plant pathogenic fungi.

Fungal diseases are considered a great problem in the production, for example *Fusarium* head blight is a global problem (Trail, 2009) and early blight, caused by *A. solani*, is a major foliar disease on solanaceous plants that limits economic production of potato and tomato (Mittelstraß et al., 2006). In this study, the broth of EPB strain SY5 had highest antibiotic activity to *B. sorokinianum*, *F. graminearum*, *F. moniliforme*, *C. musae* (*zimm.*) Hohn, *C. gloeosporiodies*, *A. solani* and *A. alternata* (*Fries*) Keissler. This result indicated that the symbiotic bacteria strain SY5 are effective against some plant pathogenic fungi and potential for using in the plant disease control.

It has already been shown that the antibiotic activity of some symbiotic bacteria strains was not noticeably affected by heating either at 60 or 121°C (Akhurst, 1982). And some studies showed that the active molecule of inhibiting the mycelium growth was mainly in the cell. After being treated with different temperature or the UV light, the culture supernatant activity of *X. bovienii*HF22 strain against *Botrytis cinerea* was not affected by the higher temperature or the UV light, but the activity of its cells was significantly decreased after incubated at 70°C for 10 min or exposure to the UV light for 0.5 h (Li et al., 2009). The EPBs could keep their primary form and antibiotic activity for two years with 1% sodium chloride solution at 10 and 23°C constant temperature (Zhang and Yang, 1995). For future evaluating the potentiality of the symbiotic bacteria strain SY5, the adversity resistance of heat stress, ultraviolet light exposure and the duration of maintenance at room temperature were examined in this study. Our results showed that the stability of the antibiotic activity against different plant pathogenic fungi was different. The antibiotic activity against *A. solani* and *A. alternata* (*Fries*) Keissler were the most stable to heat, the stability of antibiotics against *F. moniliforme*, *C. musae* (*zimm.*) Hohn, *A. solani* and *A. alternata* (*Fries*) Keissler were the best after ultraviolet light exposure for 120 min, and the antibiotic against *A. solani* was not affect by storing the strain SY5 broth at room temperature for 90 days.

In the future, a field assay is needed to prove or disprove these results. As the potential EPB for the plant disease control, the taxonomy of strain SY5 is unclear and required to be studied in the further.

In conclusion, a highly antibiotic activity EPB strain SY5 was isolated and selected. The broth of strain SY5 had highest antibiotic activity to *B. sorokinianum*, *F. graminearum*, *F. moniliforme*, *C. musae* (*zimm.*) Hohn, *C. gloeosporiodies*, *A. solani* and *A. alternata* (*Fries*) Keissler. The adversity resistance of strain SY5 showed that the stability of the antibiotic activity against different plant pathogenic fungi was different. The antibiotic activity against *A. solani* was the most stable and the inhibiting rate was not affected by treatment in a 50°C water bath for 60 min and in 100°C for 10 min, ultraviolet light exposure for 120 min and storing at room temperature for 90 days.

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