Metabolites from symbiotic bacteria of entomopathogenic nematodes have antimicrobial effects against Pythium myriotylum

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Antimicrobial effects of symbiotic bacteria from entomopathogenic nematodes for use in biorational control of *Pythium myriotylum*

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Abstract

Pythium myriotylum is a destructive soil-borne pathogen, which causes severe yield losses in many crops. The pathogen is a major economic issue for the production of ginger. Due to environmental and regulatory concerns, it is necessary to find biological alternatives to chemical pesticide for *P. myriotylum* control. In the present study, *trans*-cinnamic acid (TCA) and the fermentation broth of symbiotic bacteria from eight species of entomopathogenic nematodes alone and the combination of TCA with the fermentation broth of each symbiotic bacterium, were tested for their effects on mycelial growth and zoospore germination of *P. myriotylum*. Results showed that TCA significantly inhibited mycelial growth. Fermentation broths from seven of the eight strains of symbiotic bacteria directly inhibited mycelial growth, especially symbiotic bacteria isolated from *Steinernema feltiae* (SN strain) and *S. riobrave* (7-12 strain). Moreover, adding TCA significantly increased the extent of the inhibitory effect of mycelial growth by fermentation broths of seven tested bacteria except that associated with *S. rarum.* All bacteria fermentation broths showed inhibitory effects on zoospore germination. However, TCA alone did not show an apparent inhibitory effect on zoospore germination as it did on mycelial growth. Antimicrobial effects on mycelial growth and zoospore germination were proportional to the concentration of symbiotic bacteria isolated from *S. feltiae* (SN strain). This research provides new options for biorational control of *P. myriotylum* using TCA and symbiotic bacteria of entomopathogenic nematodes and will facilitate the sustainable production of ginger and other crops affected by this oomycete pathogens disease.

Keywords: ginger, bacterial broth, entomopathogenic nematode, biological control, *trans*-cinnamic acid

Introduction

Ginger (*Zingiber officinale* Rosc, Zingiberaceae) is a perennial monocotyledonous herb, which can be used as both a vegetable and a medicinal plant (Afzal *et al.*, 2001). It is also a cash crop for growers in many countries including China, India, Indonesia, Fiji Australia etc (Kavitha and Thomas, 2008). In 2011 the total ginger production in China, the second largest ginger producing country in the world, was 426,032 tons from 36,007 hectare (Li *et al.*, 2014). However, ginger is cultivated, which can lead to the outbreak of soil-borne diseases such as ginger blast, damping-off and anthracnose. These diseases result in severe damage and yield loss.

Soft rot (rhizome rot) is one of the most important diseases of ginger. The disease is generally caused by the pathogens *Pythium* sp. Soft rot is reported to be most destructive disease of ginger globally (Rai *et al.*, 2018). *Pythium* sp. can infect roots, collar, and succulent parts of the rhizome (Stirling *et al.*, 2009), leading to 5 to 30 % yield and even losses, up to 100 % in the case of water logging and high temperatures (Stirling *et al.*, 2009; Wang *et al.*, 2003). Once ginger fields have been infested with *Pythium* spp., the pathogens could persistent in the soil and lead to ginger soft rot in subsequent replanting (Li *et al.*, 2014). To control ginger soft rot and other soil-borne phytopathogenic diseases, farmers repeatedly apply synthetic, plant protection products to the soil, such as Mefenoxam. Mefenoxam, which causes environmental pollution, and also negatively affects soil quality, including a decrease in soil organic matter content and repression of microbial activity (Liu *et al.*, 2017). One particular species of *Pythium* species that is extremely harmful to ginger as well as various other crops is *Pythium myriotylum* (Liu *et al.*, 2017). Conceivably, a biological control approach would be a viable alternative to the use of chemical control for this oomycete pathogens disease. In this study, we explored the potential of developing a biorational tactic for *P.*

myriotylum control.

Biocontrol organisms (or their natural by-products) have shown promise in controlling various plant diseases. For example, seven bacterial isolates, belonging to *Bacillus* and *Paenibacillus*, from New Zealand soils cause zoospore germination and germ-tube growth inhibition in *Aphanomyces euteiches* (Wakelin *et al.*, 2002). A bacterial isolate, obtained from mature strawberry fruit and determined to be *Paenibacillus polymyxa* exhibited antagonistic potential against *Botrytis cinerea*, the causal agent of grey mold in strawberries (Helbig, 2001). *Fluorescent pseudomonadse* showed antagonistic activity against *Phytophthora capsici*, a major pathogen of black pepper (Tran and Kruijt, 2008).

Entomopathogenic nematodes (EPNs) are obligate and lethal parasites of insects in the natural environment (Griffin et al. 2005). Some EPN species with high virulence have being produced commercially as biological control agents against insect pests (Acharya et al., 2019). The bacterium *Xenorhabdus* spp. is a species-specific mutualist of nematodes in the genera Steinernema (Stilwell et al., 2018), and *Photorhabdus* spp. is a species-specific mutualist of that in genera of *Heterorhabditis*. The *Photorhabdus* and *Xenorhabdus* bacteria are located in the intestine of the infective juvenile nematode (IJs), which is the only free-living stage that survives outside the insect host. IJs penetrate insect hosts through natural openings or through the cuticle and release their symbiotic bacteria into the hemocoel causing septicemia, which eventually leads to host death in 48 hr. The nematodes feed upon the bacterial cells and liquefying host tissues, mature, mate and produce progeny which emerge from the insect cadaver as IJs in search of new target hosts.

Entomopathogenic nematodes have long been identified as an important biocontrol agent against

77 various above- and below-ground pests and have been fairly well characterized (Ferreira and Malan,
78 2014; Shapiro-Ilan *et al.*, 2018) whereas their symbiotic bacteria as biological control agents have
79 received less attention. Previous research has shown that the EPN's symbiotic bacteria could be
80 used in biological control. The bacteria's secondary metabolites containing toxins, lipases, proteases,
81 antibiotics and lipopolysaccharides, are complex, and can inhibit the growth of mites (Eroglu *et al.*,
82 2019) and of various microorganisms, including fungi, Gram-positive *Micrococcus*, *Staphylococcus*
83 and *Bacillus*, as well as Gram-negative bacteria (Bi *et al.*, 2018; Caldas *et al.*, 2002; Furgani *et al.*,
84 2008; Richards *et al.*, 2008; Shi *et al.*, 2017). Some of the secondary metabolites of *Photorhabdus*
85 *luminescens* subsp. *luminescens* and *P. temperata* have been examined for antibiotic activity.
86 Anthraquinone pigments and *trans*-stilbenes were determined as antibacterial, whereas *trans*-
87 stilbenes and *trans*-cinnamic acid (TCA) were defined as antimicrobial compounds (Bock *et al.*,
88 2014).

89 Exploring the EPN's bacteria (and their metabolites) for biorational control of plant pathogens has
90 received increasing attention recently, and new species and strains of EPNs/bacteria with varying
91 activity have been increasingly reported. Indeed, a wide variety of fungal and oocyte genera have
92 been suppressed using EPN bacteria or their byproducts (Shapiro-Ilan *et al.*, 2014; Hazir *et al.*,
93 2016). Although many studies have documented the antagonistic properties of bacteria associated
94 with EPNs against fungi, the potential of these bacteria to control *P. myriotylum* remains unknown.
95 In this paper, we evaluated the antimicrobial activity of EPN symbiotic bacteria against *P.*
96 *myriotylum*. Also, the addition of *trans*-cinnamic acid (TCA) to EPN symbiotic bacteria
97 fermentation broth was assessed considering its antimicrobial characters as one of main metabolites
98 of EPN symbiotic bacteria (Bock *et al.*, 2014). The aim of our study is to exploit antimicrobial

properties of bacteria associated with EPNs, and provide insight into utilizing bacterial metabolites to control *P. myriotylum* in an integrated pest management system.

Materials and Methods

Symbiotic Bacterial strains

Eight entomopathogenic nematode species were provided by Southeastern Fruit and Tree Nut Research Lab in USDA-ARS (Byron, GA) including *Steinernema carpocapsae* (Cxrd strain), *Heterorhabditis bacteriophora* (VS strain), *S. carpocapsae* (All strain), *H. bacteriophora* (HB1 strain), *S. feltiae* (SN strain), *S. rarum* (17 C&E strain), *H. indica* (HOM1 strain), and *S. riobrave* (7-12 strain). Bacteria that are species-specific and associated with the corresponding nematode isolates were used here. Details of the strains and their symbiotic bacteria were shown in Table 1.

Preparation of bacterial fermentation broth

Symbiotic bacteria from the entomopathogenic nematodes were obtained after inoculating last-instar *Galleria mellonella* with 15μL of nematode suspension (approximately 100 infective juveniles per larvae) in a 24-well cell culture plate covered with filter paper. Infected larvae were stored at 25 ℃ for 36-48 h, then, all dead larvae were surface-sterilized with 75% alcohol for 1 min. A drop of haemolymph was next added onto NBTA medium (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 25 ℃（Kaya and Stock, 1997）. Symbiotic bacteria have two variants. Primary variant symbiotic bacteria absorbs bromothymol blue in NBTA medium so the colony turns blue. Secondary variant symbiotic bacteria do not absorb bromothymol blue so the colony is red. We used only primary variant symbiotic bacteria to do the experiment. After 48 h pure colonies of the bacteria

120 were inoculated on trypticase soy yeast (TSY) medium (4% tryptic soy broth, 0.5% yeast extract)
121 at 22 ℃, 150 rpm for 36h in an incubator shaker (HNY-2102C, Tianjin Honour Instrument Co., Ltd)
122 (Ansari *et al.*, 2003). Then fermentation broth was filtrated through a 0.22 μm filter and finally
123 stored at 4℃ until experimentation.

124 **Antimicrobial effects of cell free fermentation broths of different symbiotic bacteria and TCA**

125 *Pythium myriotylum* was isolated from diseased ginger grown in Shandong province, China. The
126 isolated fungal colonies were stored at 4 ℃ on PDA medium (potato dextrose agar) and routinely
127 sub-cultured on PDA in Petri dishes (90 mm in diameter) in the dark at 27℃ until the colonies were
128 big enough to use. As indicated in previous research, *trans*-cinnamic acid (TCA) is one of the major
129 metabolic components of EPN symbiotic bacteria showing antimicrobial properties (Bock *et al.*,
130 2014) Therefore, TCA (ACROS Organics™) was evaluated as well in the present study; the product
131 was dissolved in acetone (Bock *et al.*, 2014).

132 Assessments of test solutions on oomycete growth were carried out on PDA plates, including the
133 following 17 treatments: fermentation broth of each of the eight bacteria (10% v/v) with TCA (0.2%
134 v/v), and fermentation broth of each of eight bacteria (10% v/v) without addition of TCA; TCA (0.2%
135 v/v) was also added as a single treatment (without bacterial broth). Additionally there were four
136 controls: TSY medium (10% v/v) + acetone (0.2% v/v), TSY medium (10% v/v), acetone (0.2%
137 v/v), and sterile distilled water (SDW) (10% v/v). The cell-free supernatants and TCA were
138 incorporated into PDA at 10 % (v/ v). Prior to autoclaving PDA, the amount of prescribed distilled
139 water was reduced by 10 % to allow for subsequent addition of treatment suspensions. When the
140 media cooled to 45-50 ℃, 10 % bacterial supernatant or 0.2% TCA was added and mixed

thoroughly before pouring the plates.

After the media plates had set, an oomycete colony (5 mm diameter) was inoculated in the center of each dish and incubated at 27 ℃ in the dark. After 2 days of incubation, the diameter of the oomycete colonies was measured in two perpendicular directions using the cross method (Hazir *et al.*, 2016). All experiments had 6 replications and were performed twice in time. The inhibition rate of oomycetes was calculated by the formula (The inhibition rate = 100 × (the diameter in SDW control treatment–the diameter in treatments)/the diameter in SDW control treatment).

Zoospore germination was also assessed. Methods used for zoospore isolation was as outlined in Mbarga et al. (2012) with some modifications. A single oomycete colony (5 mm diameter) was inoculated in the center of PDA medium and incubated at 27 ℃ in the dark. After 7 days of incubation, oomycete culture was sequentially mixed with 10 ml of sterile distilled water and ground using a mixer. The zoospores were then counted, adjust to 3×10^6CFU/ml, and immediately used in the bioassay. Zoospore germination assays were carried out on 24-well plates. Each well contained 100μl zoospore suspension and 800μl Potato Dextrose Broth (PDB) and the corresponding test solution. The experiment consisted of the same treatments as the aforementioned oomycete growth bioassay: fermentation broth of each of eight tested bacterial broths (100μl) + TCA (2μl); fermentation broth of each of eight tested bacterial broths (100μl); TCA (2μl); TSY medium (100μl) + acetone (2μl); TSY medium (100μl); acetone (2μl); sterile distilled water (100μl). The plates were incubated at 27 ℃. Zoospore germination was assessed after 12 h using a concave slide and light microscope. We counted the number of germinated zoospores of 100 zoospores (Abdelzaher *et al.* 1994). A zoospore was considered germinated when the germ tube length was 1.5 times as much the zoospore diameter (Plascencia-Jatomea et al. 2010). All experiments had 6 replications and were

performed twice in time. The inhibition rate of oomycete was calculated by the formula (The inhibition rate = 100 × (the number in SDW control treatment–the number in treatments)/ the number in SDW control).

Antimicrobial effects of different concentrations of *X. bovienii* fermentation broth

In the previous experiment, the fermentation broth of *X. bovienii* (the symbiotic bacteria of *S. feltiae* SN strain) exhibited a particularly promising level of antimicrobial activity considering mycelial growth and zoospore germination. Therefore, we further evaluated the antimicrobial activity of *X. bovienii* at different dose levels via mycelial growth and the zoospore germination assay. Five levels of *X. bovienii* fermentation broth were included: 0.1%, 0.5%, 1%, 5%, 10% (v/v). TSY medium was used as a control. The procedures of mycelial and zoospore preparation, as well as assessment of mycelial growth and zoospore germination were the same as described above. All experiments had 6 replications and were performed twice in time.

Statistical analyses

Before conducting the statistical analysis, the data from two trials were tested for the significant differences. For the data of two trials on the mycelial growth, the data were combined as there was no significant differences. For the data on the zoospore germination, the data were used separately, as there were significantly differences between the two trials. The antimicrobial effects on mycelial growth and zoospore germination of *P. myriotylum* were analyzed using a two-way analysis of variance, where TCA and the strains of symbiotic bacteria were used as fixed factors. Data for mycelial growth and zoospore germination in all experiments were also analyzed by one-way analysis of variance (ANOVA) to evaluate the antimicrobial effects of different solutions on *P.*

myriotylum. Means were compared by the Tukey's multiple range test ($P \leq 0.05$). Data were presented as means ± standard error. The data analysis was performed by SPSS for windows version 21. 0.

Results

Treatment effects on mycelial growth

The results of two way ANOVA showed that the main effects bacteria strain and TCA significantly impact mycelial growth and there was a significant interaction effect as well (Strain: $F_{8,222} = 409$, $p < 0.001$; TCA: $F_{1,222} = 1516$, $p < 0.001$; Strain×TCA: $F_{8,222} = 93.5$, $p < 0.001$).The treatments had significant effects on mycelial growth based on the results of one-way ANOVA ($F_{19, 239} = 365$, $P < 0.001$) (Fig.1). Results showed that TCA alone significantly inhibited mycelial growth. The maximum inhibition rate of mycelial growth (47.5%) was observed in the treatment of the combination of TCA and Xb. Significant inhibition rate of mycelial growth was observed in the TCA treatment (31.5%) as compared with three controls :TSY+Acetone（0.47%）, TSY（0.97%）, Acetone（2.15%）whereas the treatment of Xb (35.98%) and Xc (33.91%) alone had the comparable inhibitory effect as TCA (Fig. 1). Moreover, we observed that the addition of TCA significantly enhanced the effect (growth inhibition) in all bacteria except not in Xs. With regard to fermentation broth, Xs had a stronger negative influence on mycelial growth than TCA.

Treatment effects on zoospore germination

Both trials showed similar patterns of inhibition rates of zoospore germination as shown in Fig.2. The inhibition rates of zoospore germination were enhanced both by the addition of bacteria fermentation broth (Trial 1: Strain: $F_{8,102} = 1488$, $p < 0.001$; Trial 2: Strain: $F_{8,102} = 1638$, $p < 0.001$;

Fig. 2) and TCA (Trial 1: TCA: $F_{1,102} = 911$, $p = 0.001$; Trial 2: TCA: $F_{1,102} = 1059$, $p < 0.001$; Fig. 2). Xb alone showed the maxium inhibition rate of zoospore germination as compared to other treatments with bacteria alone, with 34.78% and 38.47% in trail 1 and trial 2, respectively. However, the effects TCA addition on inhibition rate of zoospore germination were dependent on the bacteria strain. Treatments had significant effects on the inhibition rate based on one way ANVOA (Trial 1: $F_{19,119} = 10.1$, $p < 0.001$; Trial 2: $F_{19,119} = 29.8$, $p < 0.001$). Bacteria fermentation broth could inhibit zoospore germination significantly, whereas TCA alone didn't show any inhibition activity with the low value of 1.02% in trial 1 and 6.76% in trial 2.

Effects of different *X. bovienii* fermentation broth dose levels of on mycelial growth and zoospore germination

The colony size of *P. myriotylum* was inversely proportional to the dose of fermentation broth of *X. bovienii* (Fig. 3) ($F_{4,59} = 217$, $p < 0.001$). The maximum value of inhibition rate (31.18%) was observed at the level of 10%. Bacteria fermentation broth, with concentrations of 0.5% and 0.1%, did not show clearly antimicrobial activity.

Similarly, the inhibition rate of zoospores germination was directly proportional to the high dose of *X. bovienii* fermentation broth(10%, 5%, 1%). whereas low dose of fermentation broth (0.5%, and 0.1%) showed the pattern of stimulating the germination of zoospores (Fig. 4) ($F_{4,59} = 60.5$, $p < 0.001$).

Discussion

Ginger soft rot is the most devastating diseases to ginger (Wang *et al.*, 2003). Control of soft rot is difficult because *Pythium* spp. can persist in the soil for years once introduced (Le *et al.*, 2014). In

the present experiment, we evaluated the antimicrobial activity of eight species of symbiotic bacteria isolated from the corresponding eight species of EPNs against *P. myriotylum* and the effects of TCA addition. Results showed that all the fermentation broths inhibited mycelial growth and zoospore germination of *P. myriotylum*, especially *Xenorhabdus bovienii*. Furthermore, we found that *X. bovienii* fermentation broth at doses of 10%, 5%, 1% significantly inhibited mycelial growth. In addition, *trans*-cinnamic acid, as one of the major bioactive ingredients of EPN symbiotic bacteria, also inhibited mycelial growth, but not zoospore germination. These results indicated that *X. bovienii* and TCA could be potentially used as a biorational solution for *P. myriotylum*. This approach could be a replacement of chemical fungicides in the suppression of soil-borne diseases caused by *P. myriotylum*, and thereby promote the sustainable production of ginger and other crops.

Previous research reported that compounds produced by *Trichoderma viride* inhibit the growth of *P. myriotylum* and other fungi (Jeerapong *et al.*, 2015). In addition, *T. harzianum* and *T. saturnisporum* also showed strong antagonism against *P. splendens* in vitro (Jeerapong *et al.*, 2015). Seed coating treatments of ginger with *Trichoderma* spp. significantly alleviated ginger soft rot disease compared to the control without *Trichoderma* spp. coating (Ram *et al.*, 2000). Singh (2011) found that *T. harzianum* was able to suppress soft rot at the same level as Ridomil. Interestingly, seeds dipped in a suspension of *Pseudomonas fluorescens* or *Bacillus* sp. combined with inoculation with mycorrhizae *Glomus* sp. during the transplantation process showed substantial suppression of soft rot in ginger (Bhai *et al.*, 2005). Shanmugam *et al.* (2013) also reported that though the efficacy of biological control agents were lower in the fields, better control was achieved when several biological control agents were incorporated together. Conceivably, *Trichoderma* spp. could be combined with the TCA, *Xenorhabdus* or *Photorhabdus* treatments for improved control.

Antimicrobial activity of symbiotic bacteria against *P. myriotylum* depends on the species of symbiotic bacteria from entomopathogenic nematodes. In the present study, *X. bovienii* exhibited the highest level of suppression of *P. myriotylum* mycelial growth as compared to other symbiotic bacteria species. In contrast, inhibition of zoospore germination did not vary as markedly among the bacteria species.

In a previous study, *X. cabanillasii* showed antifungal activity to the plant pathogen *Fusicladium carpophilum* relative to the negative control and *X. bovienii* (Hazir *et al.*, 2016). In the same paper, the supernatants of *X. bovienii* and of *Photorhabdus* spp. did not cause any suppression of growth for the plant pathogen *Monilinia fructicola* relative to the control whereas *X. cabanillasii* caused significant suppression (Hazir *et al.*, 2016). Thus, the antimicrobial activity of various symbiotic bacteria clearly varies with specific plant oomycete pathogens. Similar results were in line with (Böszörményi *et al.*, 2010) who tested the efficacy of cell-free filtrates of 18 strains of *Photorhabdus* and *Xenorhabdus* against the plant pathogenic bacteria *Erwinia amylovora* and the oomycete *Phytophthora nicotianae*. Vanitha *et al.* (2010) also observed that antimicrobial activity against *Fusarium oxysporum* (Vanilla), *Alternaria solani* (Tomato), *Sclerotium rolfsii* (Brinjal) and *Aspergillus niger* varied with the bacterial strain of symbiotic bacteria among *Xenorhabdus* spp. Given the diversity of effects found among bacterial strains and species, in future studies, we might collect more EPN species and evaluate their potential antimicrobial activity against *P. myriotylum*, especially additional strains of *S. feltiae*. With regard to *X. bovienii*, it will be beneficial to trace the antimicrobial activity of the potential antimicrobial compounds (Houard *et al.*, 2013) in their metabolites, and to explore their commercial potential to control plant disease in the field.

Trans-cinnamic acid (TCA) in combination with different strains of symbiotic bacteria showed

different antimicrobial activity effects. The compound indicated varying effects among the strains of symbiotic bacteria. TCA was discovered in the metabolites of *Photorhabdus luminescens* (Bock *et al.*, 2014). In the present study, TCA also showed clear suppression on *P. myriotylum*. Mycelial growth was about half as much as the control.

Moreover, we also found the addition of TCA to symbiotic bacteria metabolites of *X. nematophila*, *X. cabanillasii*, *X. szentirmaii* and *P. akhurstii* significantly increased their antimicrobial activity. This result is in line with a previous study which demonstrated the combination of TCA with Xs metabolites lead to lower vegetative growth of *Monilinia fructicola* as compared to either of the two treatments alone (Hazir *et al.*, 2017). Similarly, Letsididi *et al.* (2018) found that TCA could inhibit the growth of *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*. Additionally, TCA was also reported as an antibacterial compound of *Mycobacterium tuberculosis* (Chen *et al.*, 2011).

Xenorhabdus spp. and *Photorhabdus* spp. have been reported to produce various compounds besides *trans*-cinnamic acid (TCA), which shown various functionalities for agricultural and pharmaceutical purposes (Araniti *et al.*, 2018; Chen *et al.*, 2011; Letsididi *et al.*, 2018; Lupini *et al.*, 2016). With regard to plant pathogens, there is a need to find more compounds from symbiotic bacteria associated with various EPNs from natural habitats, and subsequently the antimicrobial properties of these compounds can be evaluated systemically (this should be done with other bacteria that may have suppressive potential as well).

In conclusion, we found that *X. bovienii* metabolites and TCA showed strong antimicrobial activities against *P. myriotylum*, which causes ginger soft rot and disease in other crops. The supernatant of

X. bovienii has the potential to be used in a large scale for ginger seed treatment, such as dipping the ginger seed in the supernatant during the transplant stage. The present study provides insight into a potential integrated management system for reducing plant pathogenic fungi. Further studies should be performed to clarify the function of *X. bovienii* metabolites and TCA alone, or their combination, to control *P. myriotylum*. With regard to the broad array of plant pathogenic fungi that threaten various crops, we still need to assess suitable symbiotic bacteria (associated with EPN) for possible suppression tactics. Given the regulatory status of many chemical pesticides and their threat to the environment, it is necessary to build an EPN/bacterial resource depository at a national scale or even a worldwide scale to cope with the increasing challenge of various plant pathogenic fungi and insects pests.

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Compliance with Ethical Standards

The authors declare that ethical standards have been followed and that no human participants or animals were involved in this research.

Competing interests

The authors declare that they have no competing interests.

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Table list

Table 1. The origin and abbreviation of entomopathogenic nematodes and their symbiotic bacteria

EPN	Symbiotic bacteria	Abbreviation
Steinernema feltiae (SN strain)	*Xenorhabdus bovienii*	Xb
S. carpocapsae (All strain)	*X. nematophila*	Xna
S. carpocapsae (Cxrd strain)	*X. nematophila*	Xnc
S. rarum (17 C&E strain)	*X. szentirmaii*	Xs
S. riobrave (7-12 strain)	*X. cabanillasii*	Xc
Heterorhabditis bacteriophora (VS strain)	*Photorhabdus luminescens*	Plv
H. bacteriophora (HB1 strain)	*P. luminescens*	Plh
H. indica (HOM1 strain)	*P. akhurstii*	Pa

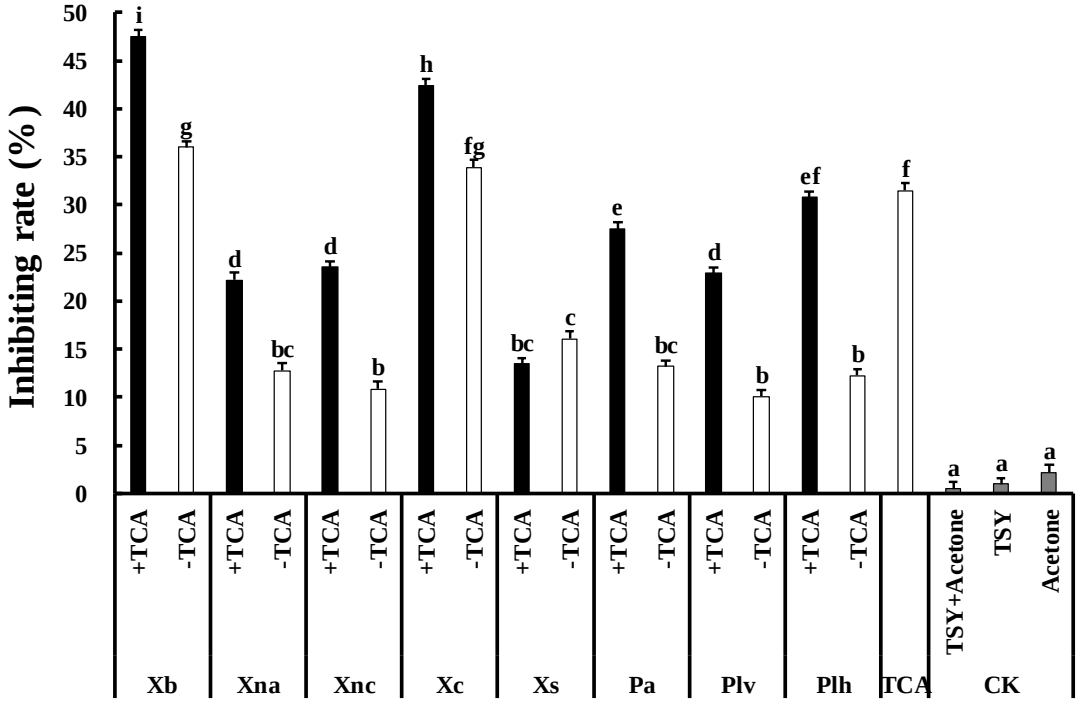
Figure caption list

Fig. 1. Effects of fermentation broth of different entomopathogenic nematode symbiotic bacteria on mycelial growth of *Pythium myriotylum*. Both fermentation broth and *trans*-cinnamic acid (TCA) inhibited the mycelial growth of *P. myriotylum*. Moreover, the addition of TCA significantly enhanced the effect (growth inhibition) in all bacteria but Xs (Xb: *Xenorhabdus bovienii*, Xna: *X. nematophila*, Xnc: *X. nematophila*, Xs: *X. szentirmaii*, Xc: *X. cabanillasii*, Plv: *Photorhabdus luminescens*, Plh: *P. luminescens* and Pa: *P. akhurstii*. TSY: trypticase soy yeast). 'TCA+' indicate the addition of TCA. 'TCA-' indicate no TCA treatment. 'CK' indicate control treatments. Different letters above bars indicate significant differences between treatments.

Fig. 2. Effects of fermentation broth of different EPN symbiotic bacteria on zoospore germination of *Pythium myriotylum*. Both fermentation broth and *trans*-cinnamic acid (TCA) could inhibit the zoospore germination of *P. myriotylum*. However, TCA and fermentation broth show antagonistic effect on inhibition of germination. 'TCA+' indicate the addition of TCA. 'TCA-' indicate no TCA treatment. 'CK' indicate control treatments. Different letters above bars indicate significant differences between treatments.

Fig. 3. Effect of *Xenorhabdus bovienii,* symbiotic bacteria (isolated from *Steinernema feltiae*) on mycelial growth of *Pythium myriotylum*. Fermentation broth at a concentration of 10%, 5% and 1%(v/v) shown antimicrobial effects against *P. myriotylum* which was inversely proportional to the dose. Different letters above bars indicate significant differences between treatments.

Fig. 4. Effect of *X. bovienii,* symbiotic bacteria (isolated from *Steinernema feltiae*) on zoospore germination of *Pythium myriotylum*. Fermentation broth at a concentration of 10%, 5% and 1%(v/v) shown antimicrobial effects against *P. myriotylum* which was inversely proportional to the dose. However, low concentration (0.5%, 0.1%) of fermentation broth could promote zoospore germination. Different letters above bars indicate significant differences between treatments.



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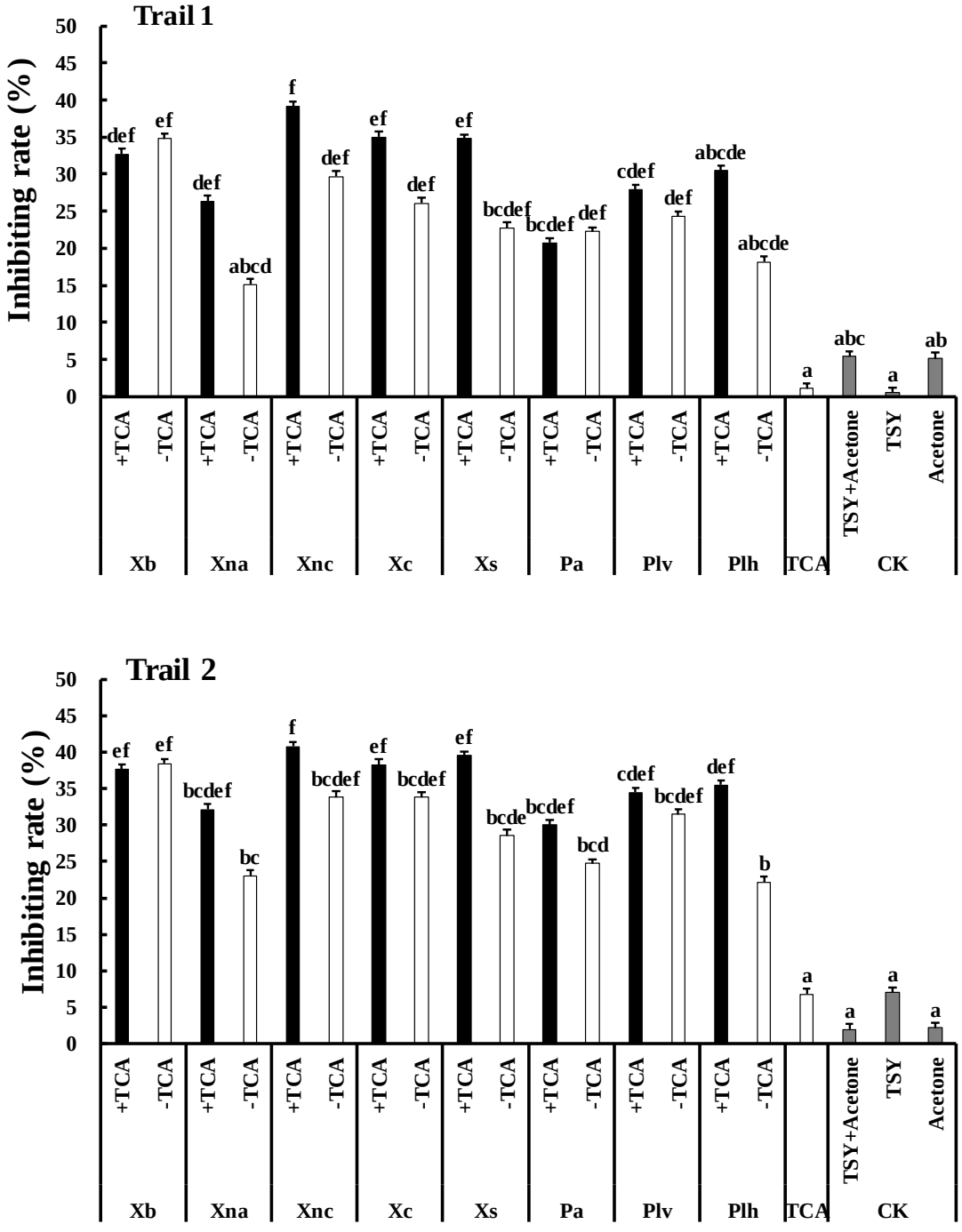
474 **Fig. 1. Effects of fermentation broth of different entomopathogenic nematode symbiotic**

475 **bacteria on mycelial growth of *Pythium myriotylum*.**



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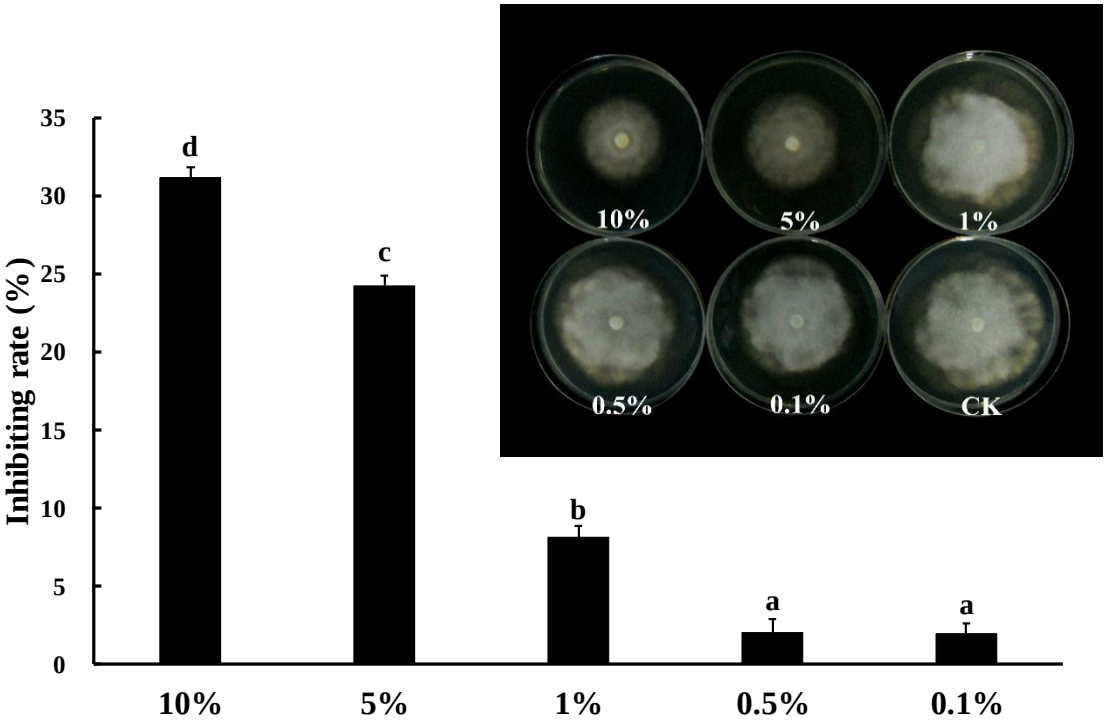

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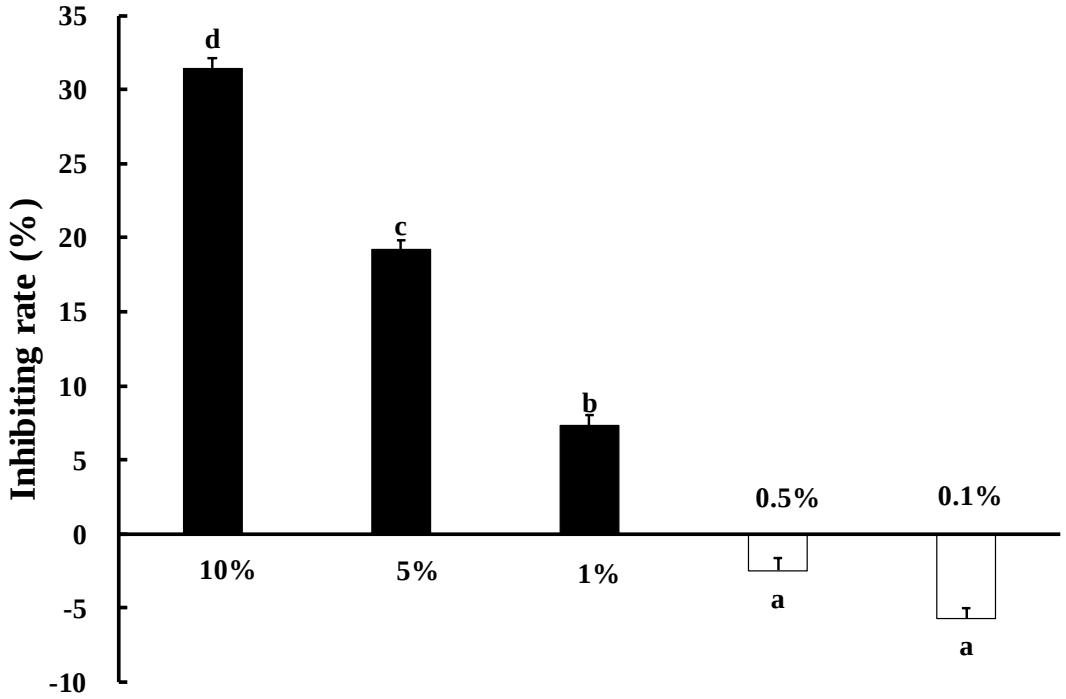

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