RESEARCH LETTER

Induction of trap formation in nematode-trapping fungi by a bacterium

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nematophagous fungi; soil bacteria; trap formation; attachment.

Abstract

Three soil bacterial strains were identified as *Chryseobacterium* sp. TFB on the basis of their 16S rRNA gene sequences. Conidia of *Arthrobotrys oligospora* produced a few mycelial traps (MT) and conidial traps (CT) when cultured with bacterial cells that they did not produce when cultured with a bacterial cell-free culture filtrate. However, co-culture of *A. oligospora* with bacterial cells and bacteria-free filtrate simultaneously induced MT and CT in large amounts. With the increased concentration of bacteria-free filtrate, the number of typical CT increased, but conidial germination was progressively inhibited. Scanning electron microscopy of *A. oligospora* co-cultured with bacteria revealed that bacterial attachment to hyphae was a prerequisite to trap formation and that bacteria-free filtrate facilitated bacterial attachments to hyphae. The results that the addition of nutrients in co-culture medium decreased the number of traps suggest that this type of trap formation may be favoured at a low nutrient status. Eight fungi tested were able to form MT and CT when co-cultured with bacterial cells and bacteria-free culture filtrate, but the abilities varied among species. This study provides novel evidence that under laboratory conditions, soil bacteria attaching to hyphae could induce traps in nematode-trapping fungi.

Introduction

Over 200 species of predacious fungi develop specific morphological structures called traps that adhere to, penetrate, kill and digest free-living nematodes in the soil (Li *et al.*, 2000). Among the nematode-trapping fungi, differentiated structures such as adhesive nets, branches and knobs as well as mechanical traps called constricting or nonconstricting rings are well known and typical of particular species (Nordbring-Hertz *et al.*, 2002). The formation of traps is very important for these fungi. These fungi thus enter the parasitic phase and capture nematodes on the surface of these structures. The traps can develop from hyphal branches and these are termed mycelial traps (MT). Alternatively, they can also form directly upon spore germination without an intermediate mycelial phase or on the germination hyphae, forming conidial traps (CT). MT can be formed either spontaneously or be induced in response to signals from the environment, including certain amino acids, valyl peptides and nemin that were secreted by host nematodes (Dijksterhuis *et al.*, 1994). CTs were formed when conidia were allowed to germinate in cow dung (Dackman & Nordbring-Hertz, 1992), fungistatic soil (Mankau, 1962), rhizosphere soil or soil extracts (Persmark & Nordbring-Hertz, 1997), and the formation of CT was believed to be a response to nutrient deprivation due to strong nutrient competition between soil microorganisms.

Fungi and bacteria coexist in a myriad of different environments. One such environment is the rhizosphere, which contains different species of bacteria and fungi in close proximity. These microorganisms influence each other's physiology and metabolism as well as the health of the plants that they might colonize (de Boer *et al.*, 2005). One study showed that several species of bacteria could influence trap formation in four nematode-trapping fungal isolates of *Dactylaria brochopaga* and *Arthrobotrys conoides*

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to trap nematode *Panagrellus silusiae* (Rucker & Zachariah, 1987). It was suggested that two substances, one produced by bacteria and one by the prey, synergistically induce trap formation. Some bacteria associated with *Arthrobotrys oligospora* could enhance *in vitro* fungal activity against the nematode and were called nematophagous fungus helper bacteria, but the mechanisms involved in the helper function were not known (Duponnois *et al.*, 1998).

In this study, three bacteria that could induce trap formation (CT and MT) in *A. oligospora* were isolated from agricultural soil. Their 16S rRNA gene sequences were used to identify these bacteria. To further understand the mechanism behind trap formation, we used a plate assay and scanning electron microscopy (SEM) technique. With these methods, we investigated the impact of bacteria on fungal trap formation. We also studied the trap formation (CT and MT) in nematode-trapping fungi by bacteria.

Materials and methods

Microorganisms and growth conditions

Bacterial strains were cultured in nutrient agar. The nematode-trapping fungi used in this study are listed in Table 1. All nematode-trapping fungi were grown at 25 °C on corn meal agar supplemented with K_2HPO_4 2 g L^{-1}. Conidia from 1–4-week cultures were used for inoculation of the experiments. Suspensions of conidia were prepared using sterile water with 0.01% Triton X-100 and used immediately. Conidial densities were adjusted to 10^6 conidia mL^{-1} in sterile water.

Table 1. List of nematode-trapping fungi used in the experiments and their infection structures

Arthrobotrys oligospora Fres. (ATCC 24927)*,†	Adhesive network (AN)
A. oligospora (1.1495)*,† (isolated from agriculture soil, Yunnan)	AN
A. oligospora (1.1435)† (isolated from cow faeces, Yunnan)	AN
A. oligospora (ZQ-85)† (isolated from grape soil, Yunnan)	AN
A. oligospora (1.12)† (isolated from Fanjing mountain, Guizhou)	AN
A. musiformis†	AN
A. dactyloides†	Constricting rings
Monacrosporium ellipsosporum†	Sticky knobs

*Used in experiments of bioassay for bacteria that could induce trap formation.

†Used in experiments of trap formation in nematode-trapping fungi by *Chryseobacterium* sp. TFB cells and its cultures simultaneously; all these isolates were kept in our collection for several years; ATCC, American Type Culture Collection.

Survey of soil bacteria capable of inducing trap formation in *A. oligospora*

A sandy agricultural soil studied previously for the presence of nematophagous fungi (Zhang *et al.*, 2005) was used. Areas of 15 m^2 of soil were selected at random and two independent rhizosphere samples were taken from each area. Each of the rhizosphere samples comprised total roots from five randomly selected wheat plants. The roots were shaken vigorously to eliminate the soil not tightly associated with roots. About 100 rhizosphere samples were taken and mixed thoroughly in a plastic bag to yield a composite sample.

One gram of the composite sample was suspended in 5.0 mL of sterile-distilled water, vortexed (1 min) and sonicated (1 min) in an ultrasonic cleaner. Soil dilution plates (10^{-5}) were prepared on nutrient agar and incubated for 7 days at 25°C. Eighty colonies of bacteria were selected at random for the ability to induce trap formation. After culturing all isolates at 25°C for 3 days in a 25-mL vial containing 10 mL nutrient broth (0.1 mg mL^{-1}, final concentration), the cultures were evaluated for trap formation. The negative controls were nutrient broth (0.1 mg mL^{-1}, final concentration) without bacteria. The first screening step for trap formation among 80 bacterial isolates was performed on only one Petri plate for each bacterium. Only the bioassay experiments for active strains were prepared in triplicate and repeated three times. This procedure was repeated three times.

Bioassay for bacteria-induced trap formation in *A. oligospora*

Trap formation was bioassayed using small Petri plates (60 mm diameter). Two *Arthrobotrys* isolates were used: *A. oligospora* (ATCC 24927) and *A. oligospora* (1.1495). Tested solutions and controls (each 3 mL) were added to Petri plates together with 200 μL of freshly harvested conidia of *A. oligospora* and incubated at 25 °C. Traps were never observed when conidia of *A. oligospora* were cultured only in the negative control media for nearly 1 month. The Petri plates were assessed 8, 16, 24 and 48 h after inoculation for the presence of traps, using an inverted microscope. Approximately 100 conidia of *A. oligospora* were scored for trap formation in each experiment.

Identification of bacteria that can induce traps in *A. oligospora*

Genomic DNA was extracted and amplified from bacteria according to the procedure described by Xu *et al.* (2003). 16S rRNA gene was amplified by PCR using TaKaRa Ex Taq (TaKaRa Biotechnology) with the following primers: A 20F (5′-GAGTTTGATCCTGGCTCAG-3′, positions 9-27) and B

1500R (5′-GTTACCTTGTTACGACTT-3′, positions 1509-1492). The PCR temperature program was 95°C for 5.5 min, followed by 35 cycles for 1 min at 94°C, 55°C for 40 s and 72°C for 2 min and with a final 10-min extension at 72°C. Following amplification, the PCR product was purified and sequenced using an ABI PRISM model 3770 DNA sequencer. Sequence was deposited in GenBank under the accession no. HQ895718. This sequence was compared to known sequences found in the GenBank database using BLAST (http://www.ncbi.nlm.nih.gov/BlAST). Multiple sequences were aligned with published sequences retrieved from EMBL using CLUSTAL_X (Thompson *et al.*, 1997) and edited via the BIOEDIT program (Hall, 1999). A phylogenetic tree was constructed on the basis of the neighbour-joining (Saitou & Nei, 1987) method; distances were estimated using MEGA version 2.1 (Kumar *et al.*, 2001). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1352 resampled datasets.

Effects of bacterial cells and its cell-free culture filtrate on the production of traps in *A. oligospora*

Preparation of bacterial cells and culture

A loop of bacterial cells from a slant culture of a fresh nutrient agar was cultivated in nutrient broth by shaking at 180 r.p.m. for 24 h at 25 °C. The fresh culture (3 mL) was placed into another Erlenmeyer flask with nutrient broth. Then they were incubated on a rotary shaker at 180 r.p.m. for 48 h at 25 °C, standardized to a density equivalent of approximately 1×10^9 CFU mL^{-1}.

Effect of bacterial cell-free culture filtrate

The bacterial cells were separated from the culture broth by centrifugation at 13 000 ***g*** for 10 min at 4 °C and the harvested supernatant was filtered through a 0.22-μM filter (Millipore UK Limited). Tested solutions containing 5%, 10%, 20%, 30% or 40% v/v cell-free culture filtrates were prepared by potato dextrose broth (PDB) dilution (1 : 50). Then these solutions were used to assay for trap formation. Controls were PDB dilutions (PDB 1 : 50) containing 5% or 40% NB (v/v). The experiments were prepared in triplicate and repeated three times.

Effects of bacterial cells without and with its cell-free culture filtrate

Bacterial cultures were centrifuged at 3000 ***g*** for 5 min at 4 °C to pellet the cells. The supernatants were removed and the bacterial cells were washed with sterile water three times. Then the cells were resuspended in PDB dilutions (PDB 1 : 50) to yield different working concentrations (0.33, 1, 1.67, 2.33, 3.0 and 3.67×10^7 CFU mL^{-1}, using dilution plating on nutrient agar). These solutions were used to bioassay for trap formation. The negative controls were PDB dilutions (PDB 1 : 50). The experiments were prepared in triplicate and repeated three times.

Bacteria cells were obtained as described above and resuspended in PDB dilutions (1 : 50) containing 20% v/v bacterial cell-free culture filtrate to yield different working concentrations (0.33, 1, 1.67, 2.33, 3.0 and 3.67×10^7 CFU mL^{-1}). These solutions were used to bioassay for trap formation. The negative controls were 20% NB (v/v) prepared by PDB dilutions (PDB 1 : 50). The experiments were prepared in triplicate and repeated three times.

Bacterial cells were resuspended to yield working concentrations of 1.67×10^7 CFU mL^{-1} in PDB dilutions (1 : 50) containing 5%, 10%, 20%, 30% or 40% v/v bacterial cell-free culture filtrates. These solutions were used to assay trap formation. Four control plates were included in each test run. Two control plates were PDB dilutions (PDB 1 : 50) containing 5% NB (v/v) without or with bacterial cells (final concentration, 1.67×10^7 CFU mL^{-1}). And the other two were PDB dilutions (PDB 1 : 50) containing 40% NB (v/v) without or with bacterial cells (final concentration, 1.67×10^7 CFU mL^{-1}). The experiments were prepared in triplicate and repeated three times.

SEM

Arthrobotrys oligospora conidia were cocultivated with bacterial cells (final concentration, 1.67×10^7 CFU mL^{-1}) containing or not containing 20% bacterial cell-free culture filtrates in PDB dilution (PDB 1 : 50). *Arthrobotrys oligospora* conidia were cocultivated (PDB 1 : 50) with bacterial cells (final concentration, 1.67×10^7 CFU mL^{-1}) in sterile water. Negative controls were PDB dilution (PDB 1 : 50) containing 20% NB (v/v) or sterile water. Fungal pellets absorbed by variable volume pipette were placed into another Petri plate and washed with 10 mL sterile water three times, mounted on a stub and coated with gold. The specimens were examined using a FEI Quanta 200 scanning electron microscope from FEI Company (Hillsboro) in the high-vacuum mode at 20 kV. Each treatment was performed in duplicate and the experiment was repeated three times.

Nutrient addition

Bacterial cells were resuspended to a working concentration of 1.67×10^7 CFU mL^{-1} prepared by sterile water or a nutrient solution consisting of PDB, a series of dilutions of PDB (1 : 5, 1 : 10, 1 : 20, 1 : 30, 1 : 50, 1 : 100, 1 : 200) containing 20% v/v bacterial cell-free culture filtrates. These solutions were used to assay trap formation. Negative controls

were PDB and PDB dilutions (1 : 5, 1 : 200) containing 20% NB (v/v).

Trap formation in nematode-trapping fungi by bacterial cells with its cell-free cultures

Trap formation was assayed using small Petri plates (60 mm diameter). Bacterial cells were resuspended to yield a working concentration of 1.67×10^7 CFU mL^{-1} prepared by PDB dilutions (PDB 1 : 50) containing 20% v/v bacterial cell-free culture filtrates. Test solutions (3 mL) were added to Petri plates together with 200 μL of freshly harvested conidia of the fungi and incubated at 25 °C. The Petri plates were assessed 24 h, 48 h, 4 days and 7 days after inoculation for the presence of traps, using an inverted microscope. Approximately 100 conidia of each strain were scored for trap formation in each experiment. Negative controls for this experiment were PDB dilutions (1 : 50) containing 20% NB (v/v).

Statistical analysis

To examine the effects of bacterial cells and its cell-free culture filtrate or nutrient addition on the production of traps in *A. oligospora*, three replicate plates per treatment were arranged in a randomized block design. Data were subjected to one-way ANOVA, followed by Duncan's multiple-range test and Thamhane's T2 (unequal variances according to a one-sample Kolmogorov–Smirnov test) at $P < 0.05$. STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES (SPSS) 11.5 software was used.

Results

Screening of soil bacteria-induced trap formation in *A. oligospora*

To screen bacteria that can induce trap formation in nematode-trapping fungi, 240 bacterial isolates from soil were screened for their ability to induce traps in *A. oligospora*. Eighteen strains showed inducing activity; three strains induced CT at an intermediate level (26–34%), but showed stable induction activity within 24 h.

Identification of bacteria capable of inducing trap formation in *A. oligospora*

Three bacterial isolates shared 99.9% 16S rRNA gene sequence similarity. Based on bacterial cell morphology and 16S rRNA gene sequences, we concluded that these isolates belonged to the same species. Sequence comparisons of 16S rRNA gene sequences with those found in GenBank indicated that three strains were closely related to the genus *Chryseobacterium*. The phylogenetic trees constructed are presented in Fig. 1. The strains most closely related to these three isolates were *Chryseobacterium indologenes* LMG 8337^T, *Chryseobacterium arthrosphaerae* CC-VM-7^T and *Chryseobacterium gleum* ATCC 35910^T, with 98.5%, 98.2% and 98.2% sequence similarity, respectively, while sequence

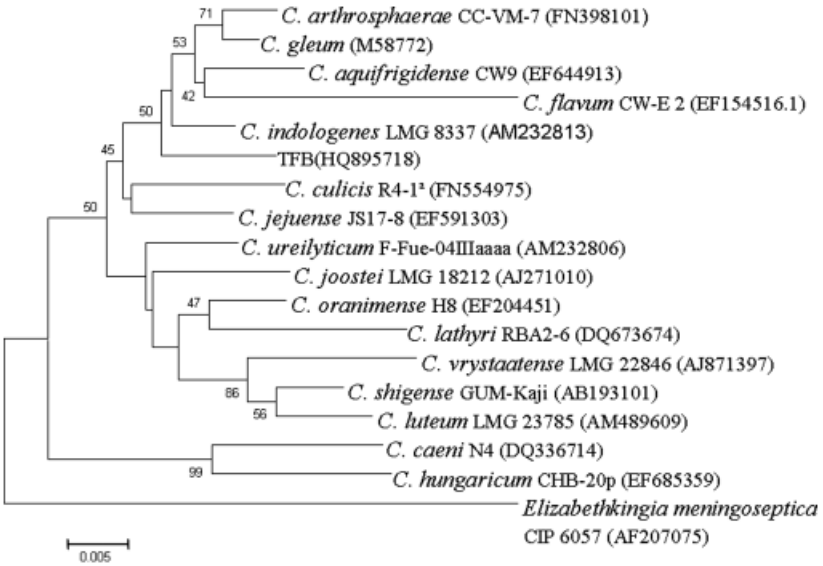

Fig. 1. Phylogenetic tree based on 16S rRNA gene (1352 bp) sequences available from the EMBL database (accession numbers in parentheses) constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the kimura-2 model) and clustering with the neighbour-joining method were performed using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1352 replications are listed as percentages at branching points (only values ≥ 40% are shown). Scale bar = 0.005 nucleotide substitutions per nucleotide position.

similarities were below 98% for all other species of the genus *Chryseobacterium*. Based on these 16S rRNA gene phylogenetic data, we suggest to name our trap-inducing isolates *Chryseobacterium* sp. TFB.

Effects of *Chryseobacterium* sp. TFB cells and its cell-free culture filtrate on the production of traps in *A. oligospora*

Effect of *Chryseobacterium* sp. TFB cell-free culture filtrate

Although a high concentration of the bacterial cell-free culture filtrate inhibited conidia germination and hyphal growth, it did not induce CT or MT in *A. oligospora* at any concentration (Supporting Information, Fig. S1). As shown in Fig. S1f, higher concentrations of bacterial cell-free supernatant inhibited the conidia germination. The cell-free supernatant also inhibited hyphal growth and caused hyphae curling (Fig. S1c–e). The surface of the hyphae looked rough (Fig. S1g).

Effects of *Chryseobacterium* sp. TFB cells without and with its cell-free culture filtrate

The conidia of *A. oligospora* co-cultured with different concentrations of *Chryseobacterium* sp. TFB cells produced a few CT and MT within 24 h, but there was no significant relationship between the percentage of traps and the concentration of bacterial cells (Fig. 2). The number of traps increased significantly ($P < 0.05$) within 24 h when the conidia of *A. oligospora* were cultured in different concentrations of *Chryseobacterium* sp. TFB cells with 20% bacterial cell-free culture filtrates (Fig. 2). The percentage of traps increased as the concentration of *Chryseobacterium* sp. TFB cells increased from 0.33 to 3.0×10^7 CFU mL^{-1} and then decreased at the highest concentration of bacterial cells of 3.67×10^7 CFU mL^{-1}. However, the highest concentrations of bacterial cells also caused conidia lysis (data not shown).

When cultured with bacterial cells (1.67×10^7 CFU mL^{-1}) in PDB dilutions (1 : 50) containing 5% bacterial cell-free filtrate, conidia of *A. oligospora* produced more MT and a few CT within 24 h (Fig. 3e–f and 4). With increased concentration of bacterial cell-free filtrates from 5% to 10%, the number of total traps, MT and CT all increased, with the number of MT increasing more than that of CT (Fig. 4). When the conidia were cultured in bacterial cells (1.67×10^9 CFU mL^{-1}) with 20% cell-free supernatant, *A. oligospora* produced 50% CT at 24 h and 90% CT at 48 h. Most traps were on the long germination hyphae while near conidia (Fig. 3n–p), and some traps formed directly upon germination with minimal or no hyphal extension (Fig. 3l and m) and the CT have several loops (Fig. 3m). With

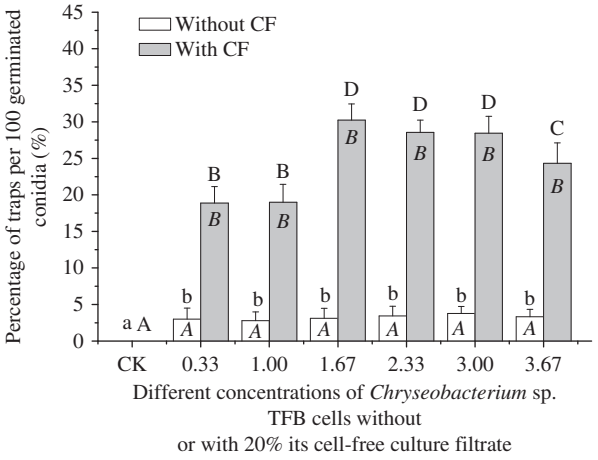

Fig. 2. Effects of different concentrations of *Chryseobacterium* sp. TFB cells ($\times 10^7$ CFU mL^{-1}) without or with 20% its cell-free filtrate (CF) on the formation of CT and MT in *Arthrobotrys oligospora*. Values are the means ± SD of nine replicate plates performed on three separate occasions. Statistically significant differences in the effects of *Chryseobacterium* sp. TFB cells without or with CF on trap formation are, respectively, denoted with a, b or A, B, C, D. Statistically significant differences between the effects of *Chryseobacterium* sp. TFB cells without CF and with CF at the same cell concentration on trap formation are denoted with *A* and *B* (Duncan's multiple-range test). CK, Control.

increased concentration of bacterial cell-free supernatant from 30% to 40%, *A. oligospora* produced more typical CT (Fig. 3h–k) and few MT (Fig. 4). Conidia germination was inhibited when cultured in bacteria with more aliquots of bacterial cell-free supernatant (data not shown). In the negative control treatment, no traps formed even when conidia of *A. oligospora* were cultured for 1 month (Fig. 3d).

Nutrient addition

With the addition of different nutrient levels to co-culture medium at the start of the experiment, the percentage of conidia germination and trap formation increased within 24 h with the decreasing nutrient (Fig. S2). However, the percentage of conidia germination as conidial and MT decreased when conidia were cultured in bacterial cells with dilution PDB (1 : 200) and sterile water.

SEM images of the *A. oligospora* hyphae

SEM observations revealed that *Chryseobacterium* sp. TFB cells attached to *A. oligospora* hyphae and traps (Fig. 5e–l) when *A. oligospora* conidia were cultured with bacterial cells (1.67×10^7 CFU mL^{-1}) containing its cell-free culture filtrates (20%) in PDB dilution (1 : 50). There were no bacterial cells that attached to *A. oligospora* hyphae when *A. oligospora* conidia were cultured with bacterial cells in sterile water or PDB dilution (1 : 50) (Fig. 5b–d). SEM results suggested that bacterial cell-free filtrates facilitated its cells

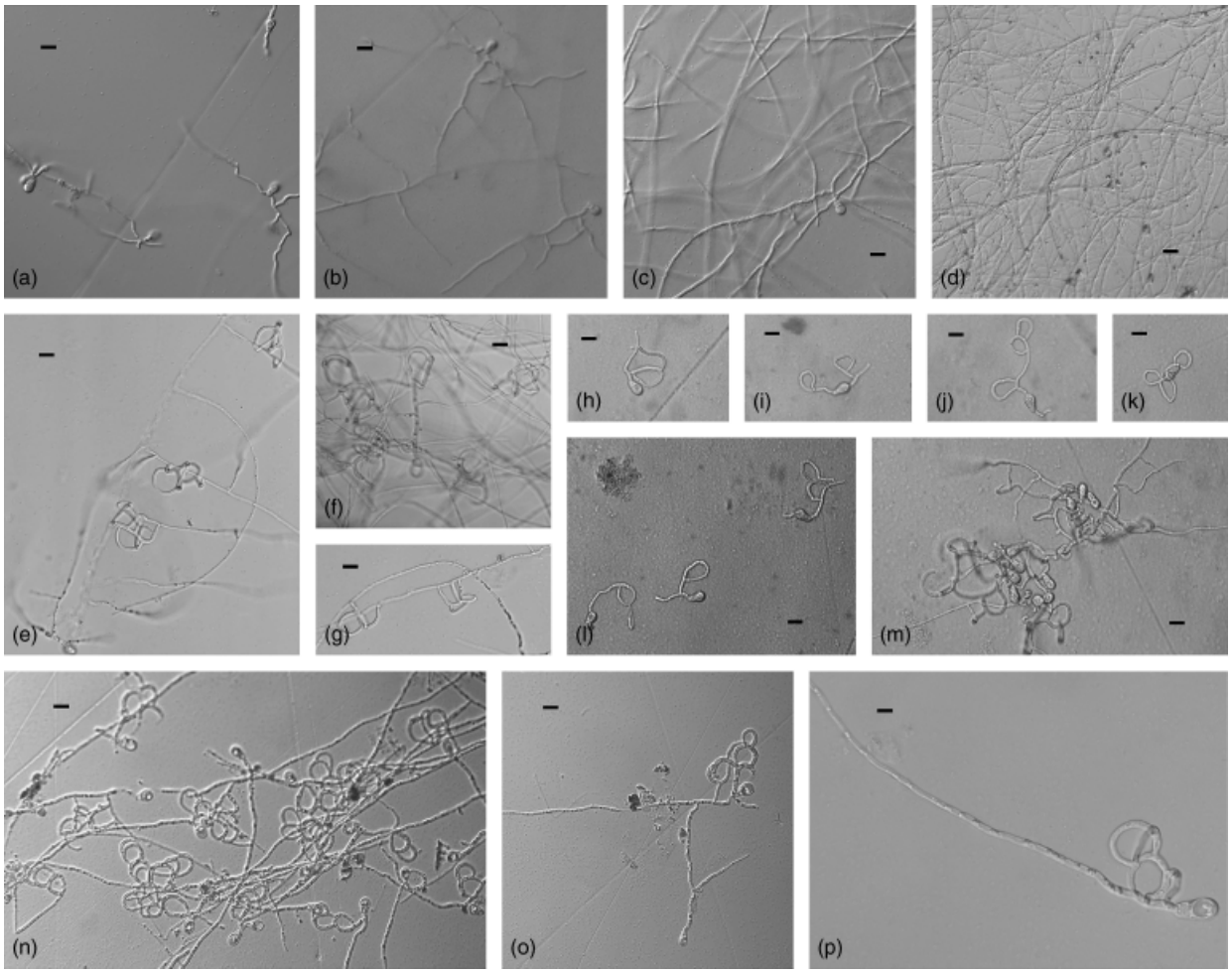

Fig. 3. Effects of *Chryseobacterium* sp. TFB cells (CsT: 1.67×10^7 CFU mL^{-1}) with different concentrations of its cell-free culture filtrate (CF) in PDB dilutions (1 : 50) on the formation of mycelial and CT in *Arthrobotrys oligospora*. (a) Conidia of *A. oligospora* germinated after incubation for 12 h; (b) normal mycelia of *A. oligospora* cultured for 24 h; (c) bushy hyphae of *A. oligospora* cultured for 48 h; (d) hyphae of *A. oligospora* cultured for 1 month; (e–g) MT of *A. oligospora* cultured with CsT cells in the presence of 5% CF for 24 h; (h–k) typical CT of *A. oligospora* cultured with CsT cells in the presence of 30% or 40% CF for 24 h; (l, m) CT of *A. oligospora* cultured with CsT cells in the presence of 20% CF for 24 h; (n–p) CT of *A. oligospora* cultured with CsT cells in the presence of 20% CF for 24 h were on the long germination hyphae.

adhering on the surface of *A. oligospora* hyphae and bacteria attached to *A. oligospora* hyphae could induce trap formation in *A. oligospora*.

Effect of *Chryseobacterium* sp. TFB cells with its cell-free supernatant on trap formation in nematode-trapping fungi

CT and MT were observed in all the nematode-trapping fungal species tested. However, the extent of trap formation differed between species. *Arthrobotrys oligospora* strains isolated from different soils could form CT and MT frequently as *A. oligospora* ATCC 24927 (data not shown). *Monacrosporium ellipsosporum* also formed many sticky knobs, most frequently at short intervals on young hyphae (data not shown). *Arthrobotrys dactyloides* developed fewer constricting rings and *Arthrobotrys musiformis* formed fewer traps than the above mentioned species, and traps were all on the long germination hyphae (data not shown).

Discussion

Several previous studies have shown that traps of the non-spontaneous trap formers are induced either by organic compounds or by nematodes (Dijksterhuis *et al.*, 1994). Jaffee *et al.* (1992) questioned the need for special trap-inducing compounds in soil as they found that more traps were produced from nematodes infected with nematode-trapping fungi when placed in soil extracts compared with those placed in a KCl solution. Persmark & Nordbring-Hertz (1997) also indicated that soil microorganisms might be involved in the formation of CT. Furthermore, the presence of bacteria

increased trap formation in four nematode-trapping fungi more than nematodes by themselves (Rucker & Zachariah, 1987). These studies indicate that bacteria play an important role in the transition of the fungi into a parasitic habit, although this transition was thought to be the result of a certain level of competition for nutrients between fungi and bacteria (Persmark & Nordbring-Hertz, 1997). Our study indicates that the formation of MT and CT in nematode-trap fungi in soil is related to specific bacteria and their metabolites. Induction was clearly due to bacterial cells with its metabolites simultaneously, as bacteria alone induced a few traps and their metabolites did not induce traps. This is the first study demonstrating soil bacteria as being responsible for MT and CT formation in nematophagous fungi.

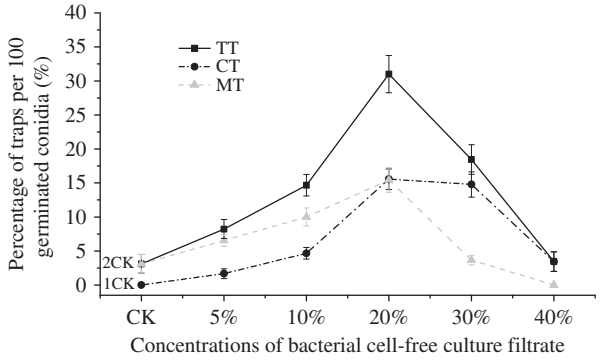

Fig. 4. Effects of bacterial cells (1.67×10^7 CFU mL^{-1}) with different concentrations of bacterial cell-free culture filtrate on the formation of CT and MT in *Arthrobotrys oligospora*. Error bars represent the SD of the mean nine replicate plates performed on three separate occasions. TT, Total traps; CK, control; 1CK, without bacterial cells; 2CK, with bacterial cells.

Trap formation in *A. oligospora* could be caused by bacterial metabolites that are released into the environment. To test whether diffusible low-molecular-weight signalling molecules triggered fungal trap formation, we treated the fungal culture with the supernatant of the bacterial culture as well as heat-inactivated bacteria. In no case was the fungal trap formation observed. Obviously, the induction of traps in fungi depends on the direct contact between the fungus and the bacterium. This assumption was unambiguously confirmed by SEM of fungal hyphae obtained from

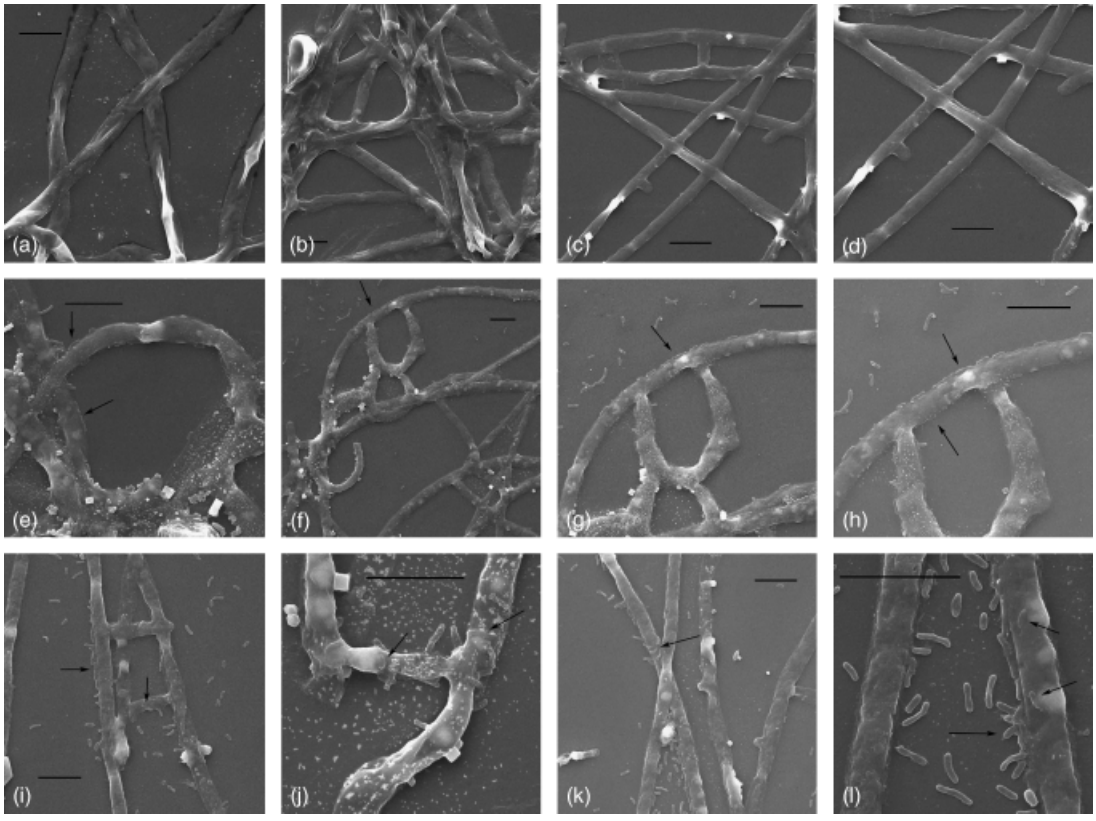

Fig. 5. SEM evaluation of interaction between *Chryseobacterium* sp. TFB (*C*sT) and *Arthrobotrys oligospora* trap formation. (a) *Arthrobotrys oligospora* conidia were cultured in PDB dilution (1 : 50) for 24 h; (b) co-culture of *A. oligospora* conidia and *C*sT cells in sterile water for 24 h, no bacteria attached to *A. oligospora* hyphae; (c, d) co-culture of *A. oligospora* conidia and *C*sT cells in PDB dilution (1 : 50) containing 20% NB for 24 h, no bacteria attached to *A. oligospora* hyphae; (e–l) Co-culture of *A. oligospora* conidia and *C*sT cells with its cell-free filtrates (20%) in PDB dilution (1 : 50) for 24 h. Bacteria attached to the traps and hyphae of *A. oligospora*. Scale bar represents 10 μm. Arrows indicate examples of *C*sT cells that attached to the hyphae and traps of *A. oligospora*. *C*sT (1.67×10^7 CFU mL^{-1}).

cocultivation. In soil, many bacteria and fungi will often occupy a shared microhabitat, called the bacterial–fungal interface (Johansson *et al.*, 2004). Traditional studies have shown the presence of bacterial cells at the interface, for example on top of fungal hyphae and spores, on mycorrhized roots and in association with fungal fruiting bodies (de Boer *et al.*, 2005). Only one study showed some bacteria associated with *A. oligospora* ORS 18692 S7 and could enhance fungal activity against the nematode, but the mechanisms were unknown (Duponnois *et al.*, 1998). The mechanisms by which *Chryseobacterium* sp. TFB-induced traps in *A. oligospora* are being investigated.

The addition of nutrients decreased the formation of MT and CT. This type of trap formation is in agreement with studies where a low nutrient status might favour the initiation of trap formation (Nordbring-Hertz, 1973, 1977; Friman *et al.*, 1985; Persmark & Nordbring-Hertz, 1997). However, very low nutrient levels could decrease the inducivenes for trap formation. It is possible that at very low nutrient levels, bacteria produce fewer metabolites that can enhance the attachment of its cell to fungal hyphae, and thus it induced fewer traps in fungi.

Nematode-trapping fungi are facultative parasites of nematodes with varying saprophytic/parasitic ability (Cooke, 1964). They may be divided into the spontaneous trap formers (in our study *A. dactyloides* and *M. ellipsosporum*), which are considered as efficient parasites, and the nonspontaneous trap formers (in our study *A. oligospora* and *A. musiformis*), which are considered as good saprophytes. The study of Persmark & Nordbring-Hertz (1997) showed that fungi with the highest saprophytic ability had the lowest capacity to form CT when cultured with soil bacteria. However, in our study, *A. oligospora* showed the highest capacity. The recent study (Warmink *et al.*, 2009) supported the viewpoint that the fungal mycosphere could indeed exert a selective pressure on particular soil bacteria. In our study, *Chryseobacterium* sp. TFB was isolated from the soil in which *A. oligospora* was the preponderant species (Zhang *et al.*, 2005). Thus, it is possible that this bacterium may be selected by *A. oligospora* and can induce traps in *A. oligospora* efficiently. We are currently examining this possibility.

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Authors' contribution

L.L. and M.M. contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Influence of *Chryseobacterium* sp. TFB cell-free filtrates (CF) on *Arthrobotrys oligospora*.
Fig. S2. Effect of nutrient addition on trap formation in *Arthrobotrys oligospora* by *Chryseobacterium* sp. TFB cells (1.67×10^7 CFU mL^{-1}) with bacterial cell-free culture filtrate (20%).

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