Journal of Agricultural Technology 2012 Vol. 8(7): 2259-2272
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Selection of efficient nematophagous fungi against root-knot nematodes in the highland cultivated area

Sumalee Mensin[1], Kasem Soytong[2], Robert J McGovern[1] and Chaiwat To-anun[1*]

[1]Department of Plant Pathology, Chiang Mai University, Chiang Mai, Thailand, [2]Biocontrol Research Unit & Mycology Section, Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok, Thailand

Sumalee Mensin, Kasem Soytong, Robert J McGovern and Chaiwat To-anun (2012) Selection of efficient nematophagous fungi against root-knot nematodes in the highland cultivated area. Journal of Agricultural Technology 8(7):2259-2272.

A number of highly virulent nematophagous fungi were recovered by soil sprinkling technique from both infested nematode plantation sand areas rich in organic matter in four provinces of Thailand. Four isolates of genus *Arthrobotrys* and two isolates of genus *Monacrosporium* selected from forty-five soil samplings damaged 90-100% second stage juveniles (J2) of root-knot nematodes (*Meloidogyne incognita*) using adhesive nets structures while two isolates, genus *Paecilomyces* and genus *Pochonia* infected 70-75% of eggs by means of appressoria. Results of morphological and molecular identification were generally concordant. The morphological and molecular data were in agreement for four fungal isolates, DLO1-001 (*Arthrobotrys oligospora*), MTI2-001 (*A. oligospora*), API3-001 (*Arthrobotrys conoides*) and MSO1-001 (*Arthrobotrys musiformis*). The conidiophore patterns and conidia classified JDI1-001 and MPI1-003 as genus *Monacrosporium*. Nevertheless, the 5.8s-ITS2-28s rDNA sequence data using ITS1 and ITS4 primers aligned them with *Arthrobotrys thaumasia*.

Key words: nematophagous fungi, biological control, highland, root-knot nematodes, *Meloidogyne incognita*, ITS

Introduction

Root-knot nematodes *(Meloidogyne* spp.) affected plants showing symptoms of stunting, wilting or yellowing including lumps or galls on roots. Control strategies for root-knot nematodes should be based on density reduction in soil through sustainable and eco-friendly approaches. Nematophagous fungi are natural enemies of nematodes and around 160 species are known in this carnivorous group (Wikipedia, 2012d). They are found in most fungal taxa: Ascomycetes and their hyphomycete anamorphs, Basidiomycetes,

---

[*] Corresponding author: To-anun Chaiwat; e-mail: chaiwat.toanun@gmail.com

Zygomycetes, Chytridiomycetes and Oomycetes (78 Steps Health Journal, 2012). These fungi can be classified into four major groups according to their infective strategies: trapping, endoparasitic, opportunistic and toxic fungi (Xue-Mei and Zhang, 2011). They use special mycelial structures to penetrate the nematode cuticle, invade and digest nematodes (Nordbring-Hertz *et al*., 2006). Accordingly, these fungi became of interest as bio-control agents against plant- and animal-parasitic nematodes. Persmark *et al*. (1996) showed that many nematophagous fungi have been found most frequently in the rhizospheres of plants. Nevertheless, these fungi have more complex relationship with their nematode hosts and ecology, since they also have an ability to live saprophytically (Ming-He, 2006). Morphological characteristics used for species identification of isolates included colony growth and culture characteristics on media, conidiophore branching pattern and arrangement including conidial morphology and quantity. These criteria were generally useful for species identification but in some cases interspecies overlap occurred. Therefore, molecular techniques are essential for confirmation of cultural and morphological species identification. The ITS region is now perhaps the most widely sequenced DNA region used for fungal identification. It has typically been most useful for molecular systematics at the species level, and even within species e.g., to identify geographic races (Vilgalys lab, 2012).

The objectives of this study were to select efficient nematophagous fungi against root-knot nematodes for highland plantation applications and to classify the genera and species of selected fungi based on morphological characteristics and molecular techniques.

Materials and methods

Collection of soil samplings and Isolation of nematophagous fungi

Randomized samples of 500 g of soil were collected from rhizospheres from infected root-knot nematode plantations and adjacent areas rich in organic material in highland areas in Thailand. Approximately 1 g of each soil sampling was sprinkled on the surface of three water agar (WA) Petri dishes containing antibiotics (0.05% streptomyc in sulphate and 0.01% chloramphenicol) together with a suspension of root-knot nematode eggs added as bait. The Petri dishes were incubated at room temperature (25-30ºC) for 3 and 5 days and then examined by microscope at low magnification for the appearance of trapped nematodes, trapping organs and conidia. Pure cultures of the fungi were made by single spore isolation technique.

In vitro predacity of nematophagous fungi against *Meloidogyne* spp.

Cultures of each fungus were grown in a 1:10 corn meal agar (CMA) medium containing antibiotics. Second stage juveniles (J2) or egg sacs were isolated from lettuce root galls and washed 5 times with sterilized distilled water. Two drops of water containing 100 J2 or eggs of *Meloidogyne incognita* was immediately inoculated into each Petri dish. Three Petri dishes served as replicates. They were kept at 25°C and the observations on trapping structures and trapped nematodes were taken at 3, 5 and 7 days under a microscope at 100×. Verification of the formation of predaceous structures and capturing of nematodes were recorded and percentages calculated.

Identification of nematophagous fungi based on morphological characteristics

The competent fungal cultures were maintained on CMA at 27ºC for 10 days; to observe morphological characteristics and slide-cultures were incubated for a week after inoculation of the fungi. The isolates were analyzed based on conidiophore branching patterns, and arrangement and mode of conidia production using the online database programs Mycobank, Index Fungorum and keys to the nematode-trapping genera of hyphomycetes and some similar genera developed by Annemarthe (no date)**.**

Identification of nematophagous fungi based on molecular techniques

DNA extraction

Each isolate of selected fungi was grown on PDA at room temperature. The mycelia were ground in liquid nitrogen with mortar and pestle to a fine power. The genomic DNA was extracted using the DNA Trap I (DNA TEC Cat NO.100-1009) according to the manufacturer’s instructions. Powdered mycelium was suspended in detergent solution (700 µl of extraction buffer), incubated at 65 °C for 60 min and put on ice box for 5 min. Extraction was with 120 µl neutralizer, mixed and put on ice box for 10 min. The cellular debris was pelleted by centrifugation for 5 min at 10,000 rpm. 1.5 ml of supernatant was mixed with 500 µl of trapping buffer and left at room temperature for 10 min. The mixture was centrifuged at 10,000 rpm for 1 min to harvest any pellets after discarding the supernatant. Samples were washed with 50 µl of washing buffer I and centrifuged at 10,000 rpm for 1 min to harvest whole pellets. The previous step was repeated with washing buffer II. The pellets were dried in an incubator at 65 °C and 10 µl of elution buffer was added before centrifugation.

The mixture was incubated at 65 °C for 30 min and centrifuged at 10,000 rpm for 5 min. The supernatant with DNA was kept at -20 °C prior to final characterization.

Determination of DNA concentration

DNA quality and quantity were determined by comparing with standard DNA intensity using an agarose gel electrophoresis protocol. A standard 1% (w/v) agarose gel prepared in Tris-Acetate-EDTA (TAE) electrophoresis buffer was used for analysis of total DNA preparations from fungal isolates and PCR amplicons. One gram of agarose powder was dissolved in 100 ml of 1X TAE buffer and microwaved for 2 min. The gel was cast with a sample slot comb. After approximately 30 min, the gel had solidified sufficiently to allow comb removal. TAE buffer was added in electrophoresis tank after submerging the gel. The samples in 6X gel-loading buffer were loaded into individual gel slots and run at 100 volts for 30 min. The gel was stained with ethidium bromide solution (10 µl /100 ml of buffer) for 10 min before viewing and photographing using a long wave UV transilluminator.

PCR amplification of the ITS region

PCR reaction and digestion of amplified fragments were performed according to the procedures of Korabecna, 2007; Esteve-Zarzoso, 1999. The 5.8s-ITS2-28s rDNA gene was amplified by PCR using the internal transcribed spacer primers ITS1 (5´TCC GTA GGT GAA CCT GCG G 3´) and ITS4 (5´TCC TCC GCT TAT TGA TAT GC 3´) (White, 1990). The PCR amplification was carried out using the GeneAmp^R PCR System 9700 (Applied Biosystems). Twenty µl of reaction mixture contained 10 ng of template DNA, 0.25 µM of each ITS1and ITS4 primer, PCR buffer which was comprised of 100 mMTris-HCL (pH 9.0), 500 mMKCl, 2.0 mM $MgCl_2$), 200 µM dNTPs and 0.6 unit of *Taq* DNA polymerase. The PCR amplification was programmed to carry out an initial denaturation step at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec and elongation at 72 °C for 7 min, followed by 1% (w/v) agarose gel electrophoresis and purification with PCR kit.

Sequencing and phylogenetic analysis

Sequences of PCR products were obtained from both strands with ITS1 and ITS4 primers using the dideoxy chain termination method. The PCR products generation was carried out with the BigDye® Terminator v3.1 cycle sequencing kit, (1st BASE, Singapore) and automated DNA sequencer following the manufacturer' s instructions. The Sequencher version4.7 software was used to assemble, edit and generate high-quality sequences. Sequence similarity analyses were performed using the Basic Local Alignment Search Tool (BLAST) in GenBank or databases of National Center for Biotechnology Information: NCBI BLAST Assembled RefSeqGenomes program (NCBI, 2012).

The multiple sequence alignment program: MAFFT version 6 and GeneDoc version 2.7 was used to align nucleotide sequences. The phylogenetic tree was obtained from data using one of three equally parsimonious trees obtained through 1,000 replications of an heuristic search with random, stepwise sequence addition by *PAUP* version 4.0b10 (Phylogenetic Analysis Using Parsimony). Additional ITS sequences of nematophgous fungi were retrieved from GenBank.

Results

Collection of soil samplings and Isolation of nematophagous fungi

One hundred and three isolates were obtained from the Chiang Mai area, two from Chiang Rai, four from Nakhonsawan and seven from Mae Hong Son. One hundred and one nematophagous fungi classified as trapping fungi, five isolates as endoparasites and ten as egg parasites were isolated from forty-five soil samplings. The genus *Arthrobotrys* sp. forming either adhesive nets or constricting rings was most commonly found (75%) followed by *Monacrosporium* sp. (12.93%) which formed non-constricting rings or adhesive knobs (stalked knob) structures, egg parasite *Paecilomyces* sp. (7.76%) and endoparsite *Meristacrum* using adhesive spores damaged J2 (4.31%) (Figure 1).

In vitro predacity of nematophagous fungi against *Meloidogyne* spp. was done. Each fungal isolate varied in their capacity to capture and kill nematodes. A few fungal isolates showed quickness in capturing nematodes. Notable, a destructive process was initiated by most isolates after 5 days. Seven fungal isolates, JDI1-001, MTI2-001, MSO1-001, MPI1-003, KJO1-003, WJI1-003 and API3-001 appeared to have high destructive capacities against root-knot nematodes. After 7 days, JDI1-001(*Monacrosporium* sp.) damaged 100% of second stage juveniles (J2) of root-knot nematodes and MTI2-001(*Arthrobotrys* sp.). MSO1-001(*Arthrobotrys* sp.), MPI1-003(*Monacrosporium* sp.) and API3-001 (*Arthrobotrys* sp.) damaged at 91.2%, 90.1% and 90.0%, of J2 nematodes respectively while KJO1-003 (*Pochonia* sp.) and WJI1-003 (*Paecilomyces* sp.) attacked 70.1 and 75.1% of eggs, respectively (Figure 2).

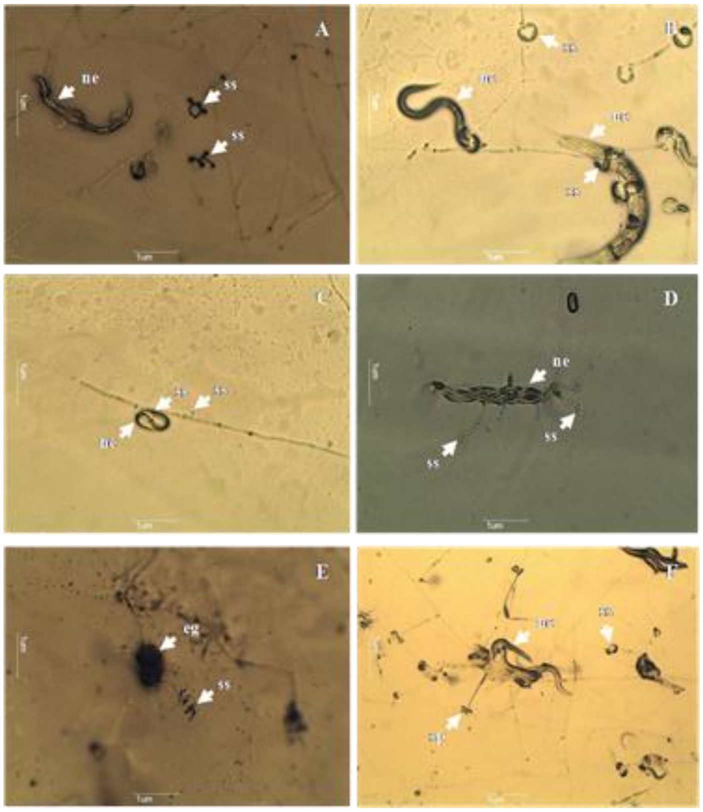


Fig. 1. Characteristics of captured nematodes(ne) or egg (eg) by special structures (ss) of some nemathophagous fungi; A. adhesive nets B. constricting rings C. adhesive knobs D. adhesive spores E. hyphal tips F. sporulation (sp)

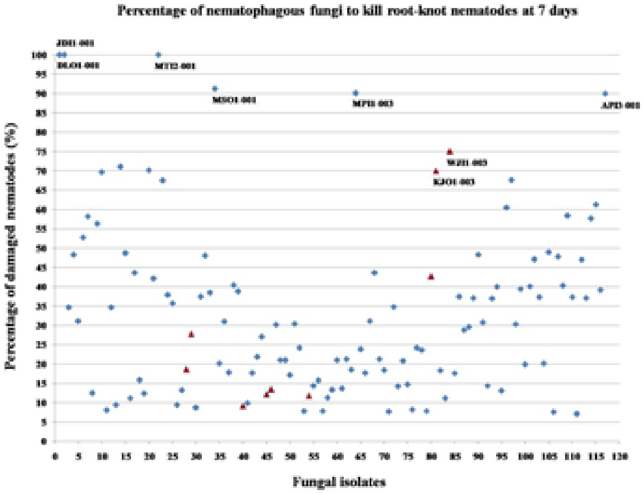


Fig. 2. Percentage of nematophagous fungi capable of killing root-knot nematodes at 7 days

● Damaging nematodes; ▲ Attacking egg of nematode

Identification of nematophagous fungi based on morphological characteristics

DLO1-001 and MTI2-001 were classified as *Arthrobotrys oligospora*. Colony textures on corn meal agar (CMA) of these fungi were fuzzy and powdery, respectively, with dirty white surface color, but the reverse side of the colonies was colorless. Mycelia grew shallowly in light concentric zones and produced a wooly pattern. Conidiophores were simple and erect, ranged in length from 200-450µm, proliferated repeatedly and sporulated heavily. Conidia were obovoidal to pyriform. Submedially, 1-2 septa were observed which sometimes showed the site of slight constrictions. The conidia of DLO1-001 were 33.10±1.41 µm long ×12.90±0.85 µm wide while conidia of MTI2-001 were 29.10±1.55 µm long×12.25±0.85 µm wide.

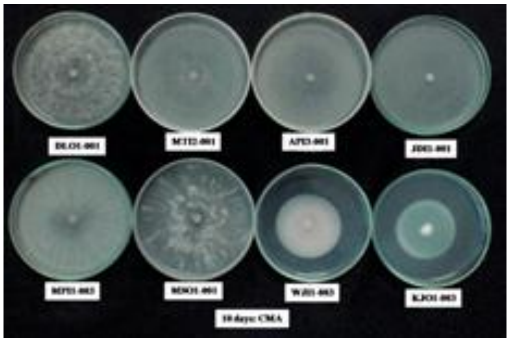
API3-001 was grouped in *Arthrobotrys conoides*. Colony texture was powdery with a dirty white color. In addition, API3-001 produced a colorless mycelial substrate and thin aerial mycelia. Conidiophores were erect, rarely branched, up to 400 µm in length, and proliferated repeatedly causing heavy conidial production. The conidiogenous heads were irregularly swollen, sometimes elongate and had short denticles. Conidia were elongate-obovoidal, with one median septum and slight constriction and measured 38.10±1.07 µm in length ×12.90±1.07 µm in width.

Monacrosporium thaumasium was the designation of JDI1-001 and MPI1-003. The surface and reverse colony color of these fungi were white and colorless, respectively. Colony textures were powdery, but differences in zonation were noted; JDI1-001 had a wooly appearance and MPI1-003 had a slight radial furrowing. Most conidiophores were simple, 150-300 µm in length, had 1-2 small perpendicular branches, and consequently these fungi produced a moderate number of conidia. Spindle-*shaped* conidia were detected on media. At their widest part conidia measured 23.15±1.09 µm and most often had two, equidistant septa.

MSO1-001 was classified as *Arthrobotrys musiformis*. Colony texture of this fungus was fuzzy and dirty white in color, but had limited growth in the center. Furthermore, the mycelial substrate was colorless and thin. Microscopically and by measurement the fungus was most similar to the genus *Arthobotrys* in that it had erect conidiophores, averaging 272.50±54.95 µm in length. However, this isolate rarely produced side branchs and proliferated subapically to produce a candelabrum-like branching system, each branch bearing a single terminal conidium. Conidia were elongate-obovoidal to ellipsoidal and 1-septate slightly below the middle. Conidia averaged 30.85±1.35 µm long×13.05±0.94 µm wide.

WJI1-003 was categorized as *Paecilomyces lilacinus*. Colonies were relatively slow-growing. Colony surface texture was velvety with a light concentric pattern consisting of numerous conidiophores and heavy sporulation. Aerial mycelium was at first white and changed to shades of light purple or sometimes was uncolored. Conidiophores were 30.25±7.34 µm in length, occasionally forming 2-4 layers of loose synnemata which had stalks with roughened thick walls. Verticillate branches with whorls of 2 - 4 phialides were often abundant. Phialides were 26-30 ±6-8 µmin length, consisting of a swollen basal portion tapering into a short distinct neck. Conidia in divergent chains were ellipsoidal to fusiform. They were smooth-walled to slightly roughened, hyaline, but purple in mass. Conidia were 3.125±0.22 µm long×3.05±0.15µm wide.

KJO1-003 was identified as *Pochonia chlamydosporia.* A creamish white and slight cottony colony was observed on CMA. Colony texture was wooly. Its aerial mycelium had shallow growth and a thin form. Conidiophores were usually prostrate and little differentiated from the vegetative hyphae, but sometimes erect and differentiated. Conidiogenous cells were phialides, tapered to a narrow tip, and were hardly visible and solitary. Conidia were transversely positioned on phialides and formed in small slimy heads. Phialides originated from prostrate hyphae, were solitary and up to five per node. Conidia were subglobose, ellipsoidal to rod-shaped, isodiametric-polyhedric, or falcate with blunt ends, 3-3.5 µm wide and mostly adhered on globose heads or chains. Colony characterization of eight nematophagous fungi are shown in Figure 3 and Table 1.



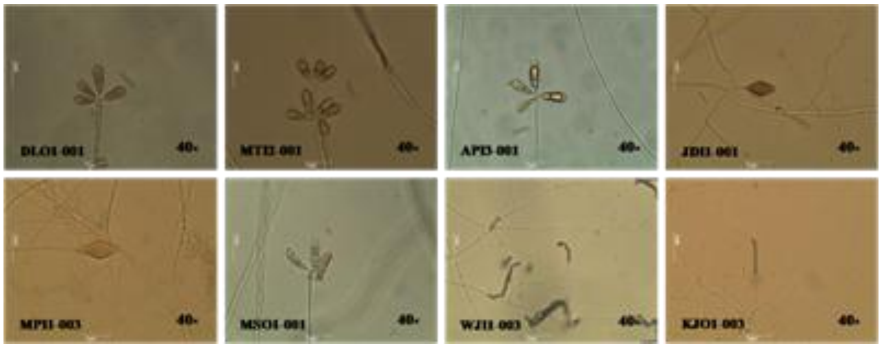


Fig. 3. Characteristics of eight fungal in colony textures, conidiophore patterns and conidia.

Table 1. Colony characterization of eight nematophagous fungi

Isolate	**Genera and Species**	**Colonydiam $^{1/}$ 5 days (cm)**	**Colony character**			**Zonation**	**Sporulation**
			Texture	Surface color	Reverse color		
DLO1-001	*Arthrobotrys oligospora*	7.5-8.0	Fuzzy	Dirty white	colorless	Light concentric zones	Heavy
MTI2-001	*Arthrobotrys oligospora*	7.5-8.0	Powdery	Dirty white	colorless	Wooly	Heavy
API3-001	*Arthrobotrys conoides*	7.5-8.0	Powdery	Dirty white	colorless	Wooly	Heavy
JDI1-001	*Monacrosporium thaumasium*	6.5-7.0	Powdery	White	colorless	Wooly	Moderate
MPI1-003	*Monacrosporium thaumasium*	6.5-7.0	Powdery	White	colorless	Slightly radially furrowed	Moderate
MSO1-001	*Arthrobotrys musiformis*	7.5-8.0	Central fuzzy	Dirty white	colorless	Slightly radially furrowed	Moderate
WJI1-003	*Paecilomyces lilacinus*	5.5-5.7	Velvety	Light purplewhite	Slightly purple	Light concentric zones	Heavy
KJO1-003	*Pochonia chlamydosporia*	5.0-5.5	Slight cottony	Creamish white	Slightly creamish	Wooly	Moderate

Identification of nematophagous fungi based on molecular techniques

The genus and species of eight isolates of nematophagous fungi were confirmed by molecular techniques. The 5.8s-ITS2-28s rDNA gene was amplified using the internal transcribed spacer primers: ITS1 and ITS4. The PCR amplified region and the PCR products ranged from 670-740 bp.

Nucleotide comparisons of these fungi using the GenBank and NCBI databases and the BLASTN 2.2.26 program indicated that DLO1-001 and MTI2-001 were *Arthrobotrys oligospora* (91% and 90% homology, respectively). Blast results identified JDI1-001 as *Arthobotrys thaumasia* with a maximum score (741 bits). MPI1-003 was identified as either *Monacrosporium thaumasium* (601 bits) or *Arthobotrys thaumasia* (597 bits) at the similar maximum identity. Five hundred and eighty nucleic acid query length of MSO1-001 was significantly aligned (91%) and identified this fungus as *Arthrobotrys musiformis*. API3-001 had highly similar sequences with *Arthrobotrys conoides*. KJO1-003 and WJI1-003 had no significant similarity with any genera or species based on molecular data (Table 2).

Table 2. The blast results of rDNA ITS sequences from nematophagous fungi and their closely

Example code[1/]	**ITS Blast result** [2/]	**Maximum Score**[3/]	**Identity**[3/]	**Gap**[3/]	**Accession#**[3/]	**Reference**[3/]
DLO1-001	*Arthrobotrys oligospora*	802	529/580 (91%)	1/580 (0%)	EU977526	Swe *et al.* (Unpublished)
MTI2-001	*Arthrobotrys oligospora*	817	565/631 (90%)	2/631 (0%)	HQ649929	Macia-Vicente *et al.* (Unpublished)
API3-001	*Arthrobotrys conoides*	817	552/616 (90%)	0/616 (0%)	JN191309	Falbo *et al.* (2011)
JDI1-001	*Arthrobotrys thaumasia*	741	509/564 (90%)	4/564 (1%)	AF106526	Hagedorn & Scholler (1999)
MPI1-003	*Monacrosporium thaumasium*	601	464/535 (87%)	4/535 (1%)	FJ380934	Kuo *et al.* (2009)
MSO1-001	*Arthrobotrys musiformis*	745	497/574 (91%)	2/574 (0%)	U51948	Liou & Tzean (1997)
WJI1-003	Non matched	-	-	-	-	-
KJO1-003	Non matched	-	-	-	-	-

Related sequences in GenBank during June 2012

[1/] Isolated from agricultural soil, Thailand

[2/] Referenced program by Zhang *et al*. (2000)

[3/] Reported first sequences producing significant alignment

Phylogenetic analysis

In this study, ITS sequences of selected fungal isolates were compared with those of 36 published nematophagous fungi; *Arthrobotrys* spp., *Monacrosporium* spp. and *Duddingtonia* sp. retrieved from GenBank. *Neurospora pannonica* and *Sordaria fimicola* were used as the out group.

Multiple sequence alignment was used to infer the maximum likelihood tree. Of the remaining 821 included characters. 244 characters were constant and 203 variable characters were parsimony-uninformative so the number of parsimony-informative included characters was 374. Nucleotide sequences based on the rDNA ITS region indicated a relationship between genotypes of some fungal isolates. Most of the selected nematophagous fungi were harmoniously clustered as blast groups. The phylogenetic relationships of Orbiliaceae which include *Arthrobotrys oligospora* (DLO1-001 and MTI2-001), *A. conoides* (API3-001) and *A. musiformis* (MSO1-001) were well defined. While the phylogenetic relationships of JDI1-001 and MPI1-003 were not clear, they had 99% BSV and nearby groups with *A. thaumasia*, *Monacrosporium thaumasium*, *M. microscaphoides*, *A. multisecun* and *M. eudermatum* as shown in Figure 4.

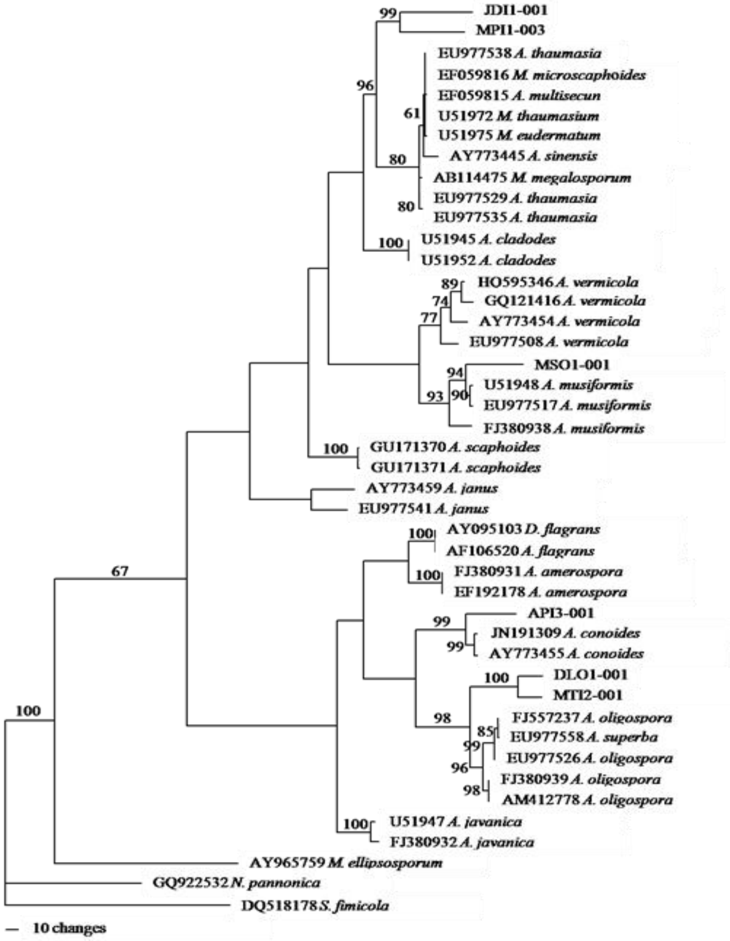


Fig. 4. One of 6 equally most parsimonious trees inferred from a heuristic search of the ITS1-5.8s-ITS2 rDNA sequences alignment of 44 isolates of *Arthrobotrys* and related genera. The size of the branches is indicated with a scale bar. Length=1,525, Cl=0.609 and Rl=0.723

Discussion

Eight isolates in three genera of competent nematophagous fungi which were collected from soil in root-knot nematode-infested areas and areas rich in organic matter from Thailand including *Arthrobotrys* sp., *Monacrosporium* sp., and *Paecilomyces* sp. Their taxonomic classification and infection structures were similar taxonomically and morphologically (infection structures) to those reported by Nordbring-Hertz *et al*. (2006), Gray (2002) and Jersys *et al*. (2009). In *in vitro* predation experiments, some isolates of the collected fungi which are nematode trapping species, endoparasites and egg parasites had low capacities to damage either second stage juveniles (J2) or eggs of root-knot nematodes. The parasitic ability of nematophagous fungi maybe related to a broad range of factors including the level of their saprophytic or absolute parasitic ability (Nordbring-Hertz *et al*., 2006) pH, moisture, organic matter, host suitability (Gray, 2002) nutrient levels, physical habitats, competitive conditions and compounds secreted by the host nematode along with the interactions in biochemical, physiological or morphological responses (Mariam, 2008). Morphological classification of the nematophagous fungi described in this research was based on such characteristics as colony diameter, culture appearance (texture, surface and reverse colouration, zonation) and colony growth rate. It is understood that the specific colony characteristics of each fungus may be different depending on a type of culture medium used as Sharma and Pandey (2010) reported Morphological identification and nucleotide comparisons at the 5.8s-ITS2-28s rDNA gene using ITS1 and ITS4 primers were in agreement for four fungal isolates of genus *Arthobotrys*. Blast results identified JDI1-001 as *Arthobotrys thaumasia*; nevertheless, conidiophore patterns and conidia classified JDI1-001 and MPI1-003 as *Monacrosporium thaumasium*. However, Index Fungorum (2012) reported that *M. thaumasium* and *A. thaumasia* (Drechsler) S. Schenck, W.B. Kendr. & Pramer, were actually synonymous *Can.* J. Bot. 55(8): 984 (1977). WJI1-003 and KJO1-003 were morphologically categorized as *Paecilomyces lilacinus* and *Pochonia chlamydosporia*, but could not be classified molecularly based on nucleotide blast format. This result may be based on the unsuitability of the sequence region and primer selection, and/or the PCR protocols used following the research of Peter and Myrian (2006) and Ciancio *et al*. (2005). The genera *Paecilomyces* and *Pochonia* include a few species that damage nematodes and they had distinct colony characteristics on most growing media so molecular identification may not be necessary.

Acknowledgement

This work was financially supported by the Thailand Research Fund (DBG5380011) and the Hitachi Scholarship Foundation.

References

Annemarthe, R. (2012). Revision of predacious Hyphomycetes in the *Dactylella-Monacrosporium* complex. Available: http://www.cbs.knaw.nl/publications/1039/content/txt_039.htm.

Ciancio, A., Loffredo, A., Paradies, F., Turturo, C. and Finetti, S.M. (2005). Detection of *Meloidogyne incognita* and *Pochonia chlamydosporia* by Fluorogenic Molecular Probes. Bulletin OEPP/EPPO Bulletin 35: 157–164.

Esteve-Zarzoso, B., Belloch, C., Uruburu, F. and Querol, A. (1999). International Journal of Systematic Bacteriology 49: pp. 329.

Gray, N.F. (2002). Ecology of nematophagous fungi: Effect of soil moisture, organic matter, pH and nematode density on distribution. Available: http://www.sciencedirect.com/science/article/pii/0038071785900173. (May 24, 2012).

Index Fungorum. (2012). Search Index Fungorum. Available: http://www.indexfungorum.org/names/Names.asp. (June 17, 2012).

Jersys, A., Leopoldo, H., Irene, M., Janaína, F.S., José, M. C.C., Regina, M.D.G.C. and Myrian, S.T. (2009). Cultural and Morphological Characterization of *Pochonia chlamydosporia* and *Lecanicillium psalliotae* Isolated From *Meloidogyne mayaguensis* eggs in Brazil. Tropical Plant Pathology 34(3):158-163.

Korabecna, M. (2007). The Variability in the Fungal Ribosomal DNA (ITS1, ITS2, and 5.8 S rRNA Gene): Its Biological Meaning and Application in Medical Mycology. Communicating Current Research and Educational Topics and Trends in Applied Microbiology pp. 783-787.

Mariam, A.A. (2008). *In vitro* Studies on Nematode Interactions with Their Antagonistic Fungi in the Rhizosphere of Various Plants. Thesis Submitted in Partial Fulfilment of the Requirements of the Degree Doctor rer. nat. of the Faculty of Forest and Environmental Sciences, Albert-Ludwigs-Universität Freiburg imBreisgau, Germany, pp. 214.

Ming-He, M., Wei-Min, C., Hong-Yan, S., Ke-Qin, Z., Chang-Qun, D. and Da-Ming, H. (2006). Heavy Metal Tolerance of Nematode-Trapping Fungi in Lead-Polluted Soils. Applied Soil Ecology 3:11-19.

Mycobank: Fungal Databases Nomenclature and Species Banks. International Mycological Association. www.mycobank.org.

National Center for Biotechnology Information: NCBI. 2012. NCBI BLAST Assembled RefSeqGenomes program. Available: http://blast.ncbi.nlm.nih.gov/. (May 29, 2012).

Nordbring-Hertz, B., Jansson, H.B. and Tunlid, A. (2006). Nematophagous Fungi. Encyclopedia of Lift Sciences, John Wiley & Sons, Ltd. www.els.net. pp. 11.

Persmark, L., Banck, A. and Jansson, H.B. (1996). Population Dynamics of Nematophagous Fungi and Nematodes in an Arable Soil: Vertical and Seasonal Fluctuations. Soil Biology and Biochemistry 28:1005–1014.

Peter, W.I. and Myrian, S.T. (2006). Identification and Taxonomy of Some Entomopathogenic *Paecilomyces* spp. (Ascomycota) Isolates Using rDNA-ITS Sequences .Genet. Mol. Biol., 29 (1), São Paulo.

Sharma, G. and Pandey, R.R. (2010). Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes. Journal of Yeast and Fungal Research 1(8):157-164.

Vilgalys lab. (2012). Conserved primer sequences for PCR amplification and sequencing from nuclear ribosomal RNA. Available: http://www.biology.duke.edu/fungi/mycolab/primers.htm. (May 27, 2012).

Wikipedia, the free encyclopedia (2012d). Nematophagous fungus. Available: http://en.wikipedia.org/wiki/Nematophagous_fungus. (May 27, 2012).

Xue-Mei, N. and Zhang, Ke-Qin. (2011). *Arthrobotrys oligospora*: A Model Organism for Understanding the Interaction Between Fungi and Nematodes. Mycology. pp 20.

78 Steps Health Journal (2012). Nematophagous Fungi. Available:http://www.78steps.com/root-knot/nematophagous-fungi.html. (May 9, 2012).

(Received 1 November 2012; accepted 30 November 1012)