AWARDS AND HONOURS

Genomic insights into the adaptation to parasitism in nematode - trapping fungi and transcriptomics during infection of *Caenorhabditis briggsae* and plant-parasitic nematodes*

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ABSTRACT: Nematode-trapping fungi are a group of soil living microorganisms that form special adhesive and mechanical trapping structures to capture and kill the nematodes. Nematode- trapping fungi belong to a family Orbiliaceae of Phylum Ascomycota. Many plant parasitic nematodes species are destructive plant pathogens which resulted in an interest to use nematode-trapping fungi as bio control agents of plant parasitic nematodes. Comparative genomics and transcriptomics were used to gain insights in to the adaptation to parasitism in nematode-trapping fungi. Sequenced the genome of *Monacrosporium haptotylum* and compared with the genome of the adhesive net forming species *Arthrobotrys oligospora*. The genome assembly contains 40.4 million base pairs and 10959 genes. Two genomic mechanisms are likely to be involved in the evolution of parasitism in nematode- trapping fungi. First, gene duplications leading to formation of novel genes and expansion of gene families resulting in a large number of species – specific genes. Many of these genes were highly expressed and upregulated during infection of *Caenorhabditis briggsae*. Second, the differential gene expression of orthologs between the two fungi during infection, suggest that the differential gene expression has been an important mechanisms for evolution of parasitism in nematode-trapping fungi. The transcriptome expressed by *Arthrobotrys oligospora*, *Monacrosporium cionopagum* and *Arthrobotrys dactyloides* during infection of root-knot nematode *Meloidogyne hapla* and sugar beet cyst nematode *Heterodera schachtii* were studied. Comparative transcriptome analysis during infection process including trapping, penetration and digestion of *Meloidogyne hapla* and *Heterodera schachtii* by nematode-trapping fungi showed that the divergence in gene expression pattern associated with fungal species was significantly larger than that related to the host nematode species. Genes that were highly expressed in all nematode-trapping fungi encoded endopeptidases, such as peptisase_S8, peptidase_M3 and aspartic proteases; cell-surface proteins containing the carbohydrate-binding domain WSC; stress response proteins; membrane transporters; transcription factors; and cell singling genes containing the Ras domain. Transcripts containing the Ricin-B lectin and Atg8 domain were also highly expressed in all nematode- trapping fungi. Differentially expressed transcripts among the fungal species encoded various lectins, such as the fungal fruit-body lectin and the D-mannose binding lectin; transcription factors; cell-signaling components; proteins containing a WSC domain; and proteins containing a DUF3129 domain. Interestingly, DUF 3129 was highly expressed in *M. cionopagum* but not expressed at all in *A. dactyloides*. Differentially expressed transcripts during infection of different host nematodes, including peptidases, WSC domain proteins, tyrosinases, and small secreted proteins with unknown function.

Keywords: *Caenorhabditis briggsae*, *Heterodera schachtii*, *Meloidogyne hapla*, nematode - trapping fungi, Transcriptomics

The term nematode-trapping fungi (NTFs) is used to describe a fascinating group of nematophagous fungi that kill and consume the free living as well as plant and animal parasitic nematodes after trapping them by their specialized adhesive and non-adhesive hyphal traps. NTFs are also known to capture other microscopic animals such as rhizopods, copepods and rotifers (Rubner, 1996). Other groups of nematophagous fungi

are endoparasites, egg and female parasites of root-knot and cyst nematodes, and toxin producing fungi (Jansson *et al.*, 1997; Jansson and Lopez-Llorca, 2001). Since the discovery of the nematode-capturing ability of *Arthrobotrys oligospora* by Zopf in 1888, NTFs have attracted the attention of mycologist across the world because of the spectacular method of capturing and infection of free living, plant and animal parasitic nematodes. Because of their ability to capture and parasitize the nematodes, they can be used as potential bio control agent of plant and animal parasitic nematodes. NTFs grow as facultative saprophytes in soil depending on their competitive saprophytic ability. These fungi switch

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over from saprophytic to predacious / parasitic phase by forming trapping devices in which nematodes are captured mechanically or by adhesion. Trapped nematodes become paralyzed (immobilized), when fungus starts to penetrate the nematode cuticle (Dijksterhuis *et al.*, 1994). The morphology of the traps of NTF differs depending on the species. Traps are basically adhesive knobs, adhesive network, adhesive branches, constricting rings and non-constricting rings. Rubner (1996) subdivided the trapping devices into seven type i.e. simple adhesive branches, unstalked adhesive knobs, stalked adhesive knobs, non-constricting rings, constricting rings, two dimensional networks and three dimensional networks. Traps of NTFs usually developed on hyphae in response to chemicals secreted by nematodes, peptides and spontaneously.

Trapping structures and induction of traps

Trapping devices are the most important infectious structures of NTFs to trap, kill and consume the nematode content to survive particularly in soil having an instance microbial competition for nutrients. NTFs usually forms traps on hyphae either spontaneously or be induced in response to signals from the environment, including peptides and other chemicals secreted by the host nematode. The ratio of carbon/nitrogen also influences the trap formation in soil environment (Dijksterhuis *et al.*, 1994). Types of the traps produced by nematode trapping fungi are given below:

Adhesive network

One of the most common traps of nematode-trapping fungi is three-dimensional adhesive network. Formation of adhesive network start with the outgrowth of lateral branches from growing hyphae, which subsequently curve and anastomose to form complex three dimensional network. Ex. *Arthrobotrys oligospora*, *Arthrobotrys conoides*, *Arthrobotrys eudermata*, *Arthrobotrys musiformis* (Fig. 1).

Adhesive hyphal branches

Adhesive hyphal branches consist of one to several elongated cells produces on the hyphae densely side by side (Fig. 1). When the adhesive branches tend to anastomose, they are called two dimensional networks. The ability of the hyphal branches to form bridges is use as a main criterion to separate *M. cionopagum* with simple adhesive branches (Fig. 1) and *M. gephyropagum* with anastomosed branches (two dimensional networks, Fig. 1). Ex. *Monacrosporium cionopagum*, *M. gephyropagum*.

Adhesive sessile and stalked knobs

Adhesive knobs are the globose or subglobose cells that is either produced on stalked attached with hypha (stalked adhesive knobs, Fig. 1) or produced sessile knob on the hyphae (unstalked adhesive knobs, Fig. 1). Ex. *Monacrosporium haptotylum*, *Monacrosporium phymatopagum*.

Fig. 1. Trapping organs of nematode – trapping fungi

Non-constricting rings

Non-constricting rings are erect lateral branches from vegetative hypha thicken and curve to form a generally three celled rings that then fused to the supporting stalk (Fig. 1).

Constricting rings

The constricting-rings are three celled and most sophisticated trapping device captures nematodes in a different way. When nematode enters into constricting rings, the three celled rings swells rapidly inwards and tightly captured the prey within 1-2 seconds (Fig. 1). Ex. *Arthrobotrys dactyloides*, *Dactylaria brochopaga*.

Habitat

Nematode-trapping fungi are soil inhabitants and found in almost all types of soil but they are most predominant in the soil having high organic matter. The most common nematode trapping fungi found in almost all types of soil are *A. eudermata* and *A. oligospora*. Only few species of NTF have been reported from aquatic and especially marine environment. Nematode-trapping fungi can grow as saprophyte in soil and as parasite using nematodes as nutrients source. The nematode- trapping fungi develop hyphal structures such as adhesive nets, adhesive knobs, adhesive branches, constricting rings and non- constricting rings to capture nematodes.

The infection process

Nematode-trapping fungi captures and parasitize nematodes in a sequence of events including adhesion

to the nematode surface or trapping, followed by penetration, digestion and growth into the nematode body (Dijksterhuis *et al.*, 1994). Species of nematode-trapping fungi forming adhesive traps capture nematodes by extracellular polymers that accumulate at the site of infection whereas constricting rings captures the nematodes by rapid swelling of ring cells (Fig. 2,3). Following trapping by adhesion or mechanical, nematode-trapping fungi form a penetration tube which pierces the nematode cuticle (Dijksterhuis *et al.*, 1994). In *Arthrobotrys oligospora*, the penetration process is a combination of the activity of hydrolytic enzymes, such as proteases, and a mechanical pressure of penetrating fungus. The cuticle of nematode consists mainly of proteins including collagen (Cox *et al.*, 1981) and various proteases have been isolated from nematophagous fungi that can hydrolyse proteins of the nematode cuticle. The penetration tube swells to form a large infection bulb in the nematode body. From the mature infection bulb, trophic hyphae develops and allow the fungus to grow and utilize the nutrients of the nematode for further growth.

Fig. 2. Steps of nematode infection by nematode – trapping fungi. (a) Capturing of free-living nematode by adhesive trap of nematode trapping fungus ($\times 350$), (b) Capturing of *Meloidogyne hapla* by constricting ring of *A. dactyloides* ($\times 350$), (c) Growth of fungal hyphae of *A. dactyloides* in the nematode by after consuming the content of nematode body ($\times 150$)

Fig. 3. (a) Adhesive network of *A. oligospora* ($\times 200$), (b) Trapping of *Heterodera schachtii* by adhesive network of *A. oligospora* ($\times 150$), (c) Trapping of *Panagrellus redivivus* by adhesive network of *A. oligospora* ($\times 100$), (d) Adhesive stalked knob of *M. haptotylum* ($\times 125$), (e) Trapping of *M. hapla* by adhesive knob of *M. haptotylum* ($\times 100$), (f) Trapping and penetration of *M. hapla* by adhesive knob of *M. haptotylum* ($\times 125$), (g) Adhesive branch of *M. cionopagum* ($\times 450$), (h) Trapping of *M. hapla* by adhesive branch of *M. cionopagum* ($\times 160$), (i) Trapping of *H. schachtii* by adhesive branch of *M. cionopagum* ($\times 225$), (j) Constricting rings of *A. dactyloides* ($\times 200$), (k) Trapping of *H. schachtii* by constricting ring of *A. dactyloides* ($\times 170$), (l) Trapping of *M. hapla* by constricting ring of *A. dactyloides* ($\times 170$)

Growth and parasitic ability of nematode-trapping fungi in fungistatic environment of soil

Disease suppressing potential of any nematode-trapping fungi in nematode infested soil depends on the successful germination of conidia and formation of specialized traps to capture the nematodes. Several workers reported that presence of fungistatic factor in soil are inhibitory to the growth of fungi. Therefore, the ability of nematode trapping fungi to overcome the soil fungistasis is of paramount importance for the bio control potential of these fungi. Some species of NTFs also form traps directly on the conidium or small germlings of conidia (conidial trap) in response to soil fungistasis, animal dung

Fig. 4. (a) Conidia of *Dactylaria brochopaga* (x550), (b) Conidial trap of *D. brochopaga* (x300), (c) Trap on the germ tube of *D. brochopaga* (x250), (d) Conidia of *A. dactyloides* (x570), (e) Conidial trap of *A. dactyloides* (x650), (f) Trapping and killing of soil nematodes by conidial trap of *A. dactyloides* (x170)

and nematodes. These conidial traps are depends on the capturing and killing of the nematodes for subsequent growth and survival. Dackman and Nordbring-Hertz (1992) called conidial traps as survival structure to overcome fungistasis. *Arthrobotrys dactyloides*, *Monacrosporium gephyrophagum* (Parsmark and Nordbring-Hertz, 1997) and *Dactylaria brochopaga* frequently form conidial traps on water agar disk in vicinity to soil and trap the nematodes (Fig. 3). *Arthrobotrys oligospora* also form frequent conidial traps in close contact to cow dung (Dackman and Nordbring-Hertz, 1992). The ability of *D. brochopaga* and *A. dactyloides* to form the conidial traps in the vicinity of soil and trapping of soil nematodes (Fig. 4) indicates that this fungus has enormous potential to reduce the nematodes in soil (Singh, 2010; Kumar *et al.*, 2015).

Bio-control potential of nematode trapping fungi against root knot nematodes

Some species of nematode-trapping fungi are potential biocontrol agents of plant parasitic nematodes. Application of mass culture of *Arthrobotrys dactyloides* and *Dactylaria brochopaga* grown on barley substrates by method developed by Kumar *et al.* (2005) was found effective against the root-knot disease of tomato and rice. The number of root knots, females, egg masses and second stage juveniles of root knot nematodes were found to reduce by the application of colony forming units of *A. dactyloides* and *D. brochopaga* in tomato and rice (Kumar and Singh, 2006; Singh *et al.*, 2007).

Genome sequence and general genomic features of *Monacrosporium hayotylum*

To know the genomic mechanism responsible for adaptation of parasitism in nematode trapping fungi,

Table 1. General features of genome of *M. haptotylum* and *A. oligospora*

Features	<i>M. haptotylum</i>	<i>A. oligospora</i>
Size (MB)	40.4	40.07
Protein coding genes	10,599	11,497
Genes encoding secreted proteins	1666	1568
Protein with P fam domain	7455	7555
Virulence associated proteins	1161	1132

genome of the adhesive knob forming fungus *Monacrosporium haptotylum* was sequenced to 28 fold coverage using 454 pyrosequencing and compared with the available genome sequence of *Arthrobotrys oligospora* (Yang *et al.*, 2011). The genome size of *M. haptotylum* was estimated to 40.4 Mb and protein-coding genes to 10,959 which are more or less similar to the numbers for *A. oligospora* (Table 1). Transposable elements (TEs) were identified in *M. haptotylum* and *A. oligospora*. Tandem duplications of the genes in families were identified in *M. haptotylum*. In total, 147 duplicated pairs consisting of 272 genes were identified as being located in tandem positions. The proportion of genes encoding secreted proteins in *M. haptotylum* was estimated to be 1,666 (Table 1). Identified 1,161 proteins in *M. haptotylum* and 1,132 proteins in *A. oligospora* that are similar to proteins in the Pathogen-host interaction protein database (PHI-base database), particularly from plant pathogens. PHI-base contains a collection of experimentally verified pathogenicity, virulence and effector genes from fungi, oomycetes and bacteria (Winnenburg *et al.*, 2006).

Role of gene family expansions in adaptation of parasitism in nematode-trapping fungi

Gene families are groups of genes descended from a common ancestor that retain similar sequences and often similar functions (Dayhoff, 1976). Expansion of lineage-specific genes as a result of the differential duplication and deletion of genomic regions has been shown to provide selective advantages and contribute to adaptation in a variety of organisms, including fungi (Lespinet *et al.*, 2002). The gene family expansions and contractions in the genomes of *M. haptotylum* and *A. oligospora* by analyzing the gene family evolution by comparing the genome of 16 fungi were studied. The change in size of 848 gene families of *M. haptotylum* of which 326 (38.4%) gene families were expanded and 522 (61.6%) contracted. Out of 806 gene families that changed in *A. oligospora*, 362 (44.9%) were expanded and 444 (55.1%) were contracted. The lowest proportion of gene families expansion among the ascomyceteous fungi was found in *Neurospora crassa* branch (9%) and the highest in *Metarhizium anisopliae* (52.6%). The expansion of gene families of the nematode-trapping fungi was similar to *Magnaporthea oryzae* (38.0%) inciting sheath blight of rice and higher than *Metarhizium acridum* (26.9%, Table 2). The nematode-trapping fungi shared more gene families being expanded between plant pathogenic fungi as compared to insect – or animal

Table 2. Expanded gene families in nematode trapping fungi and some Ascomycota

<i>Neurospora crassa</i>	9%
<i>Metarhizium anisopliae</i>	52.6%
<i>Magnaporthe oryzae</i>	38.0%
Nematode-trapping fungi	38.4%
<i>Metarhizium acridium</i>	26.9%

– pathogenic fungi. 28 expanded gene families were found shared between nematode-trapping fungi and plant pathogens. The nematode-trapping fungi and insect pathogens shared fewer numbers of expanded gene families and the nematode and animal pathogens the least (9 and 2 families, respectively).

A comparative analysis of gene families and the expanded Pfam domain families revealed strong similarities. The expansion of gene families in *M. haptotylum* and *A. oligospora* was found matched with all 25 expanded Pfam domains. This indicates that the expanded Pfam domain families are important for the evolution of the two nematode-trapping species (Meerupati *et al.*, 2013).

Identification of core, lineage- and species-specific genes of *M. haptotylum* and *A. oligospora*

Comparative genomic analysis of *M. haptotylum* and *A. oligospora* showed that these two nematode-trapping fungi contained a large number of lineage-specific and species-specific genes. We found that the around 62% genes of *M. haptotylum* and *A. oligospora* were shared by other fungi; around 20% of the genes were shared between *M. haptotylum* and *A. oligospora* and up to 16% of the genes were unique for each fungal genome. The lineage-specific and species-specific groups were enriched for genes involved in transcription, cytoskeleton, cell wall/membrane/envelope biogenesis, secretion and signal transduction whereas the core regions contained homologs of genes involved in metabolism and energy production (Meerupati *et al.*, 2013).

Gene expression in *M. haptotylum* during infection of *Caenorhabditis briggsae*

Gene expression related with nematophagous phase of *M. haptotylum* was studied by inoculation of L1 larvae of the nematode *C. briggsae* to the adhesive knobs (traps) of *M. haptotylum*. After 4 hours of infection, approximately 98% *C. briggsae* were trapped and killed. A significant enrichment of species-specific genes was found in the cohort of the ten-fold upregulated genes. Of the 10% highly expressed genes during infection of *C. briggsae* by *M. haptotylum* (1,069 genes), 117 were more than ten-fold upregulated. In total, 38 of these 117 infection-regulated genes were specific for the *M. haptotylum* lineage, 15 were unique for the *M. haptotylum* and *A. oligospora* lineage and 64 common to other fungi (core genes). These 117 infection related genes were enriched in species - specific genes encoding secreted proteins, small secreted proteins (SSPs) and genes identified to

belongs to expanded gene families. In total, 36 of the 117 regulated genes (31%) were from expanded Pfam domain families. The expanded Pfam domain families were primarily encoded by the core set of genes and they contained the DUF3129, WSC, tyrosinase, mucin and peptidase S8 families (Meerupati *et al.*, 2013). Twenty-eight of the highly infection-regulated genes showed sequence similarities to proteins in the PHI-base, including the members of the DUF3129 Pfam domain family that encode the gas1 proteins of the rice blast fungus *Magnaporthe oryzae* (Xue *et al.*, 2002), an extracellular cutinase (PBC1) from a plant pathogen (Li *et al.*, 2003), a tetraspanin homolog from *Colletotrichum lindemuthianum* (Veneault-Fourrey, *et al.*, 2006), and *RBT4* from *Candida albicans* (Braun *et al.*, 2000).

Comparative transcriptomics

The transcriptome of *M. haptotylum* and *A. oligospora* expressed during the infection of *C. briggsae* was compared to know the difference between these two fungi. Analysis of the sequences of the 10% most highly expressed genes in *M. haptotylum* (which included 1,069 genes) showed that these were slightly enriched with secreted proteins. Among them, 200 proteins (19%) were predicted to be secreted proteins of which 84 were SSPs. In the transcriptome of *A. oligospora*, seventy-two secreted proteins were identified among the 10% most highly expressed genes (in total 1,145 genes) of which 10 were SSPs. Genes from expanded Pfam domain families were highly expressed in both fungi, including the subtilisin (peptidase S8), tyrosinase, CFEM, DUF3129 (gas1), WSC and GLEYA families. Detailed analysis of the expression of genes shared between the two nematode-trapping fungi was performed. In total, 2,599 (32%) of the 8,121 ortholog pairs showed significant difference in fold change (q-value<0.01) between the two fungi. The orthologs displaying the largest differences in expression values, that is, those that were more than 10-fold up- or down-regulated in the pairwise comparison between *M. haptotylum* and *A. oligospora*, were enriched with genes encoding secreted proteins, including many lineage-specific genes and SSPs. Several expanded Pfam domain families were also enriched among the differentially expressed genes (Meerupati *et al.*, 2013).

Mechanisms of Adaptation of parasitism in nematode-trapping fungi

Two genomic mechanisms are likely to have been of major importance during the evolution of parasitism in nematode-trapping fungi:

- Formation of novel genes through gene duplication leading to the expansion of gene families resulting in a large number of species-specific genes. Many of species-specific genes were highly expressed and upregulated during infection of nematodes.
- Differential expression of orthologous genes between *M. haptotylum* and *A. oligospora* during

early stage of infection suggests that differential gene expression has been an important mechanism for the evolution of parasitism in nematode trapping fungi.

Commonly expressed genes of nematode trapping fungi during infection of plant parasitic nematodes

Transcriptome of *Arthrobotrys oligospora* (adhesive net forming fungus), *Monacrosporium cionopagum* (adhesive branch forming fungus) and *Arthrobotrys dactyloides* (Constricting ring forming fungus) were studied during infection of *Meloidogyne hapla* (root-knot nematode) and *Heterodera schachtii* (sugar beet cyst nematode). The commonly expressed genes of *A. dactyloides*, *A. oligospora* and *M. cionopagum* during infection of *Meloidogyne hapla* and *Heterodera schachtii* were subtilisin (peptidase_S8), aspartyl proteases, CFEM (a fungal specific cysteine-rich domain that is found in some proteins with proposed roles in fungal pathogenesis) (Kulkarni *et al.*, 2003), and the carbohydrate-binding WSC domain (Andersson *et al.*, 2013; 2014; Meerupati *et al.*, 2013), Pfam domain RicinB_lectin_2 (ribosome-inactivating proteins), proteins involved in fungal stress response, cell signaling, organization of the cytoskeleton, vesicular transport and membrane transport, as well as several families of calcium-binding proteins and transcription factors. Domains of enzymes and proteins involved in the carbon, energy and amino-acid metabolism and protein synthesis were also highly expressed by nematode trapping fungi. Furthermore, transcripts containing the Atg8 domain (an essential protein in autophagic pathway) were highly expressed in *A. dactyloides*, *A. oligospora* and *M. cionopagum*. *A. oligospora* has 52 genes containing the peptidase_S8 domain (Meerupati *et al.*, 2013). However, only one transcript containing the peptidase_S8 domain was identified among the highly expressed transcripts in each library of *A. oligospora* infecting *M. hapla* and *A. oligospora* infecting *H. schachtii* (Andersson *et al.*, 2014). The other cDNA libraries contained also only one transcript with the peptidase_S8 domain among the top 500 expressed genes. Stress proteins were highly expressed in all nematode trapping fungi. They included heat-shock proteins and chaperones such as DnaJ, HSP70 and HSP90; glutathione S-transferases; and antioxidant enzymes such as thioredoxin and catalase.

Interspecific variation in gene expression

Differentially expressed transcripts among the fungal species encoded peptidases (peptidase_M1 and peptidase_M24), lectins (FB_lectin, B_lectin, RicinB_lectin_2), tyrosinase, transcription factors, cell-signaling components, Atg8, various stress-response proteins, proteins containing the WSC domain and the DUF3129 domain (domain of unknown function). The DUF3129 is found in the gas1 protein of the *Magnaporthe grisea* that participate in appressorial penetration and lesion formation during infection of rice (Quest *et al.*, 2013). It was interesting to observe that DUF3129 was highly expressed in *M. cionopagum* whereas this domain

was not expressed at all in *A. dactyloides*. DUF3129 was identified in twelve transcripts among the Top500 most expressed transcripts in the library of *M. cionopagum* infecting *H. schachtii*. During *A. oligospora* infections, this domain was identified in one transcript in *A. oligospora* infecting *M. hapla* and in four transcripts in *A. oligospora* infecting *H. schachtii* among the Top500 most expressed transcripts (Andersson *et al.*, 2014). DUF3129 is an expanded gene family in nematode-trapping fungi and both *M. haptotylum* and *A. oligospora* have 33 genes encoding this domain (Meerupati *et al.*, 2013). Seventeen of these genes were found among the 10% most expressed genes during nematode infection by *M. haptotylum* while only two were among the 10% most expressed genes during nematode infection by *A. oligospora* (Meerupati *et al.*, 2013). Accordingly, the DUF3129 domain is highly expressed during infection among the species that form adhesive branches and adhesive knobs. Further studies are needed to investigate the function of the DUF3129 domain in the nematode-trapping fungi during infection. Transcripts encoding the fungal fruit-body lectin (FB_lectin) and the D-mannose binding lectin (B_lectin) were only highly expressed in *A. oligospora* (adhesive net forming fungus) but not in *A. dactyloides* (Constricting rings forming fungus) and *M. cionopagum* (Adhesive branch forming fungus).

Virulence associated transcripts

A BLAST search of the top 500 transcripts in each library of nematode trapping fungi infecting host nematode species was conducted in the pathogen-host interaction protein database (PHI-base). In total, 97 unique PHI-base genes were identified. Among the 97 identified PHI-base genes, 15 were highly expressed by *A. oligospora*, *A. dactyloides* and *M. cionopagum* during infection of plant parasitic nematodes. Virulence associated proteins expressed by nematode-trapping fungi included antioxidant, calcium binding protein, cell signaling (Pkinase), cell signalling (Ras), DEAD-box RNA helicase, membrane transport (Cation transport ATPase (P-type) family, morphogenesis (Serine/threonine-protein phosphatase), non-specific DNA-binding proteins (HMG box), stress response (FKBP-type peptidyl-prolyl cistrans isomerase), stress response (Peptidylprolyl isomerase), transcription (Repressor Tup1), Woronin body major protein (Andersson, *et al.*, 2014). The PHI-base genes that differ in expression between the nematode-trapping fungi included aspartic proteases and the gas 1 and gas 2 proteins of *M. grisea* (Xue *et al.*, 2000) that contains the DUF3129 domain (domain of unknown function).

Nematode host-specific gene expression

Gene expression in *A. oligospora* during the infection of *M. hapla* versus *H. schachtii* showed that a majority of the genes had similar expression levels. 105 genes were at least 5-fold upregulated during trapping and infection of *M. hapla* compared to trapping and infection of *H. schachtii* and 65 genes were at least 5-fold upregulated in *A. oligospora* during infection of *H. schachtii* compared

to *M. hapla*. Genes predicted to encode secreted proteins were enriched among the differentially expressed genes in both nematodes. The differentially expressed genes encoding proteins with a predicted secretion signal were further characterized. They included peptidases, glucosidases and several gene families that were expanded in the genomes of nematode-trapping fungi such as DUF3129, WSC and tyrosinase (Meerupati *et al.*, 2013). Five of the in total 22 secreted differentially expressed genes were assigned as small secreted proteins (SSPs) with unknown function (Andersson *et al.*, 2014).

CONCLUSION

The importance of nematode-trapping fungi arises from their potential use as biological control agent of plant- and animal- parasitic nematodes. In order to understand the evolution of parasitism in nematode trapping fungi, genomics, and transcriptomics were studied. By sequencing the genome of an adhesive knob forming fungus *M. haptotylum* and by analyzing comparative transcriptomics to the adhesive trap forming fungus *A. oligospora*, the gene duplication followed by functional diversification has played a major role in the evolution of parasitic nature of nematode trapping fungi. The differential regulation of orthologous genes has been an important mechanism for the evolution of parasitism in the nematode-trapping fungi in response to the nematode host. Comparative transcriptome analysis showed that the divergence in gene expression between the species of nematode trapping fungi was significantly larger than that related to the plant-parasitic nematode host. A core set of transcripts being highly expressed by all three fungi during infection of plant parasitic nematodes included subtilisins, aspartic proteases and proteins containing the CFEM domain. These genes were also found highly expressed by *A. oligospora* and *M. haptotylum* during infection of *C. briggsae*. Also within this core set, a putative nematotoxic protein was identified, the Ricin-B lectin. A more variable set of transcripts in the fungal species was also identified. A small group of genes was identified showing differential expression depending on the plant parasitic nematode host. This group was enriched in genes encoding secreted proteins and also included several gene families that were expanded in the genomes of nematode-trapping fungi. Knowledge of the genomic interspecific variation in the trapping mechanisms and host preferences will be useful for researchers for identification of more potent nematode-trapping fungi for management of plant parasitic nematodes. *A. dactyloides* and *D. brochopaga* were observed to form conidial traps and kill the nematodes in soil which indicates that conidia based formulations of these fungi could be used as potential product for application in root-knot infested soil for management of plant-parasitic nematodes.

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