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# *Arthrobotrys oligospora*: a model organism for understanding the interaction between fungi and nematodes

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*Arthrobotrys oligospora*, a predacious fungus of nematodes, has been very useful in understanding the relationship between nematophagous fungi and their nematode hosts. *Arthrobotrys oligospora* is by far the most common nematode-trapping fungus with the characteristic ability of forming adhesive trapping nets once in contact with nematodes. This review highlights the versatility and development of *A. oligospora* as a system to identify and characterize the ecology and biology of nematode-trapping fungi. Using *A. oligospora*, advances in our knowledge of nematophagous fungi have been made through the discovery of special traits and virulence determinants involved in the pathogenic process, or by creating new ways of presenting these factors to the target nematodes. We argue for an increased role for *A. oligospora* in complementing other model systems in biological control research.

**Keywords:** *Arthrobotrys oligospora*; nematode-trapping fungi; nematophagous fungi; nematode; biocontrol

## Introduction

*Arthrobotrys oligospora* Fres. 1852, the first recognized nematode-trapping fungus (Zopf 1888), is the most commonly isolated and by far the most abundant nematode-trapping fungus in the environment (Duddington, 1954; Farrell et al., 2006; Jaffee, 2004; Mekhtieva et al., 1980; Persmark et al., 1996; Satchuthananthavale and Cooke, 1967; Wachira et al., 2009). More than 120 years of intense basic and applied research on *A. oligospora* has contributed not only to the development of this species as a potential biological control agent, but also other advancements across a broader scientific context. For example, the finding that *A. oligospora* is capable of paralyzing the nematodes by producing a chemical substance, nematotoxin, has provided fundamental insights for the field of nematodetoxic fungi (Olthof and Estey, 1963). Numerous experiments on *A. oligospora* have made this species a popular model system for studying many aspects of nematophagous fungal biology, ranging from morphogenesis to pathogenesis. These studies have addressed a range of biological questions, especially on the interactions between fungi and nematodes, and such knowledge has benefited application of nematophagous fungi as potential biological control agents.

Thanks to the scientific attention given to this species over the past decades and improvements in the new methods and technologies, we have now obtained much information about microbial pathogenic factors, from morphology to molecular mechanisms. In this review, we survey the past and current states of *A. oligospora* research, and provide a brief account of knowledge of the interaction between fungi and nematodes. Future research priorities and goals are forwarded.

## History of *A. oligospora* – saprobic and predatory fungus with a special trap structure

*Arthrobotrys oligospora* was first collected in Europe, and characterized by Fresenius in 1852 (Fresenius, 1852). Its dominant life style was originally thought to be that of a saprobe, obtaining nutrients from decaying organic substrates. In 1870, Woronin (1870) found that *A. oligospora* could produce a specialized network structure (Figure 1), formed by an erect lateral branch growing from a vegetative hypha, curving to fuse with the parent hypha and developing more loops exterior to the original loop or on the parent hypha. He also observed that the conidiophores of *A. oligospora* could directly develop into a complex network structure. However, the function of the special structure remained unknown. In 1876, Sorokin (1876) reported that a ring from *A. oligospora* could infect nematodes, but he mistook the ring as its conidia and assumed that *A. oligospora* was a parasitic fungus. Zopf (1888) gave a detailed description of

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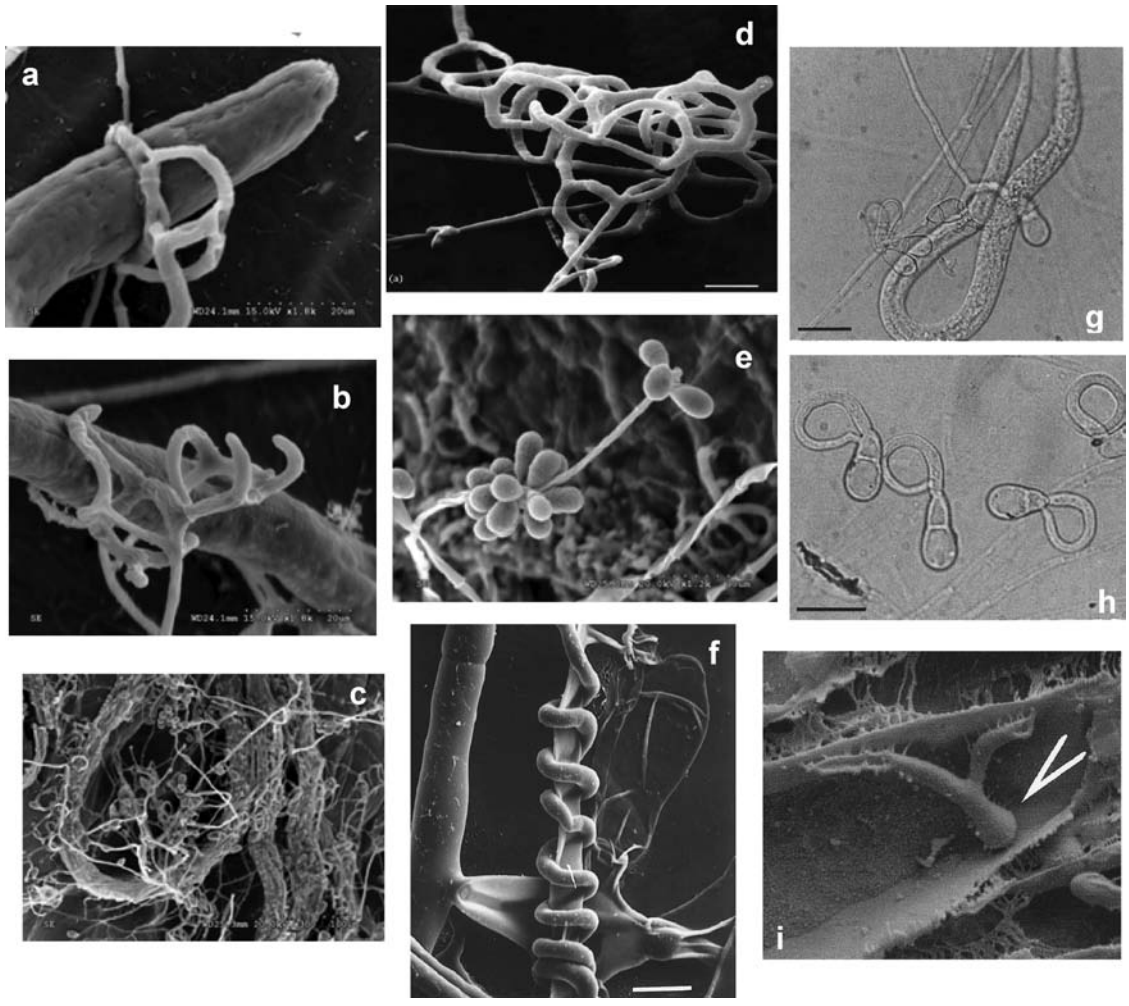


Figure 1. Morphological and structural diversities of *A. oligospora.* (a)–(c): Nematode captured by adhesive trap; (d) adhesive network, reproduced from Nordbring-Hertz (2004); (e) spores; (f) mycoparasite coiling around a hypha of *Rhizoctonia solani*, reproduced from Nordbring-Hertz (2004); (g) a nematode captured by conidial trap; (h) conidial traps, reproduced from Nordbring-Hertz et al. (1995); (i) appressorium (indicated by the white arrow) during the early colonization of barley roots, reproduced from Nordbring-Hertz (2004).

nematode-trapping process of the network structure of *A. oligospora*. He demonstrated that the specialized mycelial structure trapped nematodes, penetrated the cuticle of the worm, grew mycelia inside the prey and digested the nematodes' contents. Soon after host death, hyphae emerged from the cadaver and produced conidiophores and conidia. In addition, he also mentioned that the formation of trapping devices only occurred in a nutrient-deficient culture. These findings laid the groundwork for establishing the predaceous activity of *A. oligospora* using a special mycelia network and heralded the identification of a new mode of fungi–nematode interaction, which opened up a new perspective for the emerging field of nematode–fungi ecology. Drechsler (1933a,b) developed a new method for cultivating nematode-predating fungi by putting some minced infected plant root on agar. This simple method contributed greatly to the discovery of new nematophagous fungi (Drechsler, 1933a,b) and led to a rapid increase in the discovery of nematophagous fungi during the following 20 years. Drechsler (1934) also found that *A. oligospora* was often isolated and could produce a bulbous structure soon after capturing nematodes. From this internal appressorium, trophic hyphae grew throughout the worms. He attributed the death of the nematode to the partial severance of its body by the infection bulb of the fungus (Drechsler, 1934). Duddington (1954) carried out a survey of nematode-destroying fungi in the soil from arable land infested with potato root nematodes or cereal root nematodes, which had caused serious loss to these crops. He found that *A. oligospora* was much more prevalent than the other nematophagous fungi. This work not only attracted much attention on exploring the biological functions and infection mechanism of this fascinating fungus, but also provided the stimulus for an entire era of research on using nematophagous fungi as potential biological control agents against parasitic nematodes.

**Ecological and biological characteristics of *A. oligospora***

Ecological surveys have indicated that *A. oligospora* is by far the most broadly distributed and most frequently isolated nematode-trapping fungus in the environment (Duddington, 1954; Persmark et al., 1996; Persmark and Jansson, 1997; Peterson and Katznelson, 1964; Yan et al., 2007). It has been recorded from Asia, Africa, North and South America and Australasia, and is capable of growing in many environments, including almost all types of natural soil, animal faeces, surface waters and heavily polluted substrates. Its broad distribution suggests its immense ability to adapt and grow in diverse environmental conditions (Mo et al., 2008; Saxena, 2008; Wachira et al., 2009).

*Arthrobotrys oligospora* can grow in close association with the rhizosphere of agricultural crops and plants. It is very common in the upper 30-cm soil layer, while below 40 cm few samples have been found (Persmark et al., 1996). The density of the fungus was slightly higher in the rhizosphere than in root-free soil and different crops showed different influences on the numbers of this species in the rhizosphere. Peterson (1964) used *A. oligospora* as an index to test the occurrence of nematode-trapping fungi in the rhizosphere of wheat and soybean, and found a greater abundance of the fungus in the soybean than wheat rhizosphere or root-free soil. The proposed reason was that the soybean rhizosphere soil contained more nematodes. Further evidence came from pot and field experiments in which the rhizosphere effects of pea, barley and white mustard on nematophagous fungi were investigated. The pea rhizosphere had a significantly higher number of nematode-trapping taxa, containing up to 19 times more individuals than root-free soil, while the barley rhizosphere had a similar density of individuals as root-free soil. The author suggested that this could be due to the higher density of nematodes in the pea rhizosphere than in root-free soil (6–290 times higher). The nematode density in the barley rhizosphere was 3–13 times higher than root-free soil (Persmark and Jansson, 1997). Another interesting finding showed that a decrease in the density of nematodes only affected the number of endoparasitic nematophagous fungi. The population declined in a positive correlation with the number of nematodes, while the number of the saprobic nematode-trapping species, including *A. oligospora*, remained relatively constant (Persmark et al., 1996).

It is widely accepted that the zone around the root abounds with organisms, including plant parasitic and free-living nematodes. *Arthrobotrys oligospora,* in particular, is thought to be a 'facultative' trapper of nematodes, using them as a nitrogen source, but decomposes organic matter as its carbon and energy source (Barron, 1992; Cooke, 1963). Thus, there is a potentially underappreciated food chain in the soil with plant roots providing food to nematodes which serve as a nitrogen source for nematode-trapping fungi that subsequently reproduce (Barron, 2003). Bordallo et al. (2002), however, found that *A. oligospora* could colonize the rhizosphere of axenic barley and tomato (i.e. without nematodes), and grew chemotactically towards the root surface. The fungus grew inter- and intracellularly in barley and tomato roots, colonizing the epidermis and cortex regions but never penetrating the vascular tissues of plant roots. In addition, it also induced plant defense reactions without harming the development of the plants. The induced defense might render the plants more resistant to plant parasitic nematodes and/or other pathogens. This result is consistent with the carnivorous *A. oligospora* having an endophytic lifestyle (Jansson and Lopez-Llorca, 2004). The fact that *A. oligospora* could colonize the rhizosphere may have significant implications for its suitability as a biocontrol agent.

*Arthrobotrys oligospora* is part of a complex food chain in the coastal soils of the Bodega Marine Reserve (Farrell et al., 2006; Nguyen et al., 2007). Jaffee and Strong (2005) suggested that bush lupines, ghost moths, isopods and an entomopathogenic (insect-parasitic) nematode contributed to the abundance of *A. oligospora* at Bodega Marine Reserve. They observed that the strongest numerical response in the presence of moths was by *A. oligospora* and this fungus frequently 'bloomed', with its population density increasing 10–100 times and sometimes exceeding 10,000 propagules/g of soil. They also found that the growths of three other nematode-trapping fungi producing adhesive nets similar to *A. oligospora* were not enhanced by nematode-parasitized insects. One possible explanation for this result was that those three species could not increase and were even suppressed because their niches overlapped with that of *A. oligospora* (Farrell et al., 2006; Koppenhöer et al., 1996).

*Arthrobotrys oligospora* is often found in faeces (Bird and Herd, 1995; Hay et al., 1997a; Saumell et al., 1999; Sayers and Sweeney, 2005; Su et al., 2007). The most frequently studied strain ATCC 24927 was collected from the dung of livestock (Nordbring-Hertz, 1977a). Every year, there are many reports on strains of *A. oligospora* isolated from faeces in different regions which are then screened for their biological control activities against parasitic gastrointestinal nematodes. A time-series examination of sheep faeces indentified a total of 123 fungal species from the 120 sheep faecal samples deposited on pastures in the Mata Region of Minas Gerais State (Brazil) over four seasons. Among these, *A. oligospora* and *Monacrosporium eudermatum* (Drechsler) Subram. 1964 were the most common predatory species (Saumell and Padilha, 2000; Saumell et al., 1999). In recent studies, we investigated the effects of season and altitude on the occurrence of nematode-trapping fungi in cattle faeces in west Yunnan. Seventeen nematode-trapping species were collected from 660 samples on three plateau pastures of different altitudes

(Su et al., 2007). Along with *Monacrosporium ellipsosporum* (Preuss) R.C. Cooke & C.H. Dickinson, 1965, *A. oligospora* was among the predominant species throughout the year. These results were consistent with the observations that *A. oligospora* is one of the most predominant predatory fungi, capable of colonizing faeces from both wild and domestic animals in different regions, and could be found during different seasons and in different ecological pastures (Grønvold et al., 1993b; Hay et al., 1997a; Sayers and Sweeney, 2005). At present, there are several hypotheses for the entry of nematode-trapping fungi into animal dung. One is the splash dispersal of conidia from the air or soil. Another is anthophilous dispersal by soil invertebrates, such as mites (Hay et al., 1997a,b). It is also possible that nematode-trapping fungi in the upper layers of soil and foliage could grow into the dung from underneath (Hay et al., 1997b; Persmark et al., 1996) and that the fungal traps could be carried into the dung by soil infected by nematodes (Nansen et al., 1988).

*Arthrobotrys oligospora* has also been isolated repeatedly from aquatic environments, including marine water (Alias et al., 1995), and the physico-chemical parameters of waters were found to have little effect on its distribution (Kiziewicz and Czeczuga, 2003). Because there is no published account that directly demonstrates the active growth of *A. oligospora* in natural waters, its presence in water might be due to secondary deposition as runoffs from terrestrial environments.

*Arthrobotrys oligospora* is often referred to as a 'biological indicator' of nematodes in mushroom growth facilities. It is well known to mushroom growers that the mass appearance of the fungus is associated with the infection of nematodes at the same sites (Cayrol, 1979; Grewal and Sohi, 1988).

*Arthrobotrys oligospora* has been isolated from contaminated environments, including heavy metal-polluted mines and soil sprayed with fungicides and nematicides (Kiziewicz and Czeczuga, 2003). Our recent survey of the diversity of nematode-trapping fungi in the soil from lead mines in Yunnan Province, where Pb concentrations ranged from 132 to 13,380 mg/kg, revealed that *A. oligospora* is the most frequently isolated nematode-trapping fungus. Strains of this species isolated from Pb-contaminated soil showed greater tolerance to Pb than those from Pb-free soil (Mo et al., 2006, 2008). This indicates that strains thriving in heavy metal-contaminated ecosystems may have increased resistance to toxic metals, demonstrating their strong evolutionary adaptation potential. Evaluations of the susceptibility of *A. oligospora* to fungicides and nematicides frequently used for controlling plant pathogen and nematodes in soil demonstrated that *A. oligospora* was the most resistant nematode-trapping fungus (Persson et al., 1990; Tunlid et al., 1999).

In summary, *A. oligospora* can grow in diverse environments, including soils, around plant roots and faeces of animals, and is especially widespread in nematode-infested environments. *Arthrobotrys oligospora* has a high saprobic ability and efficiently utilizes a diversity of carbohydrates. The apparent ubiquity and biological characters of *A. oligospora* are strongly correlated with its infective abilities towards nematodes. Large increases in resident nematodes usually result in large increases in *A. oligospora* propagules, and the responses of *A. oligospora* to nematodes are generally much stronger than those of other trapping fungi. These characteristics have made *A. oligospora* an excellent candidate from which to develop an effective biocontrol agent (Bird and Herd, 1995; Chandrawathani et al., 1998; Grønvold et al., 1993a; Hashmi and Connan, 1989; Jaffee, 2004; Yan et al., 2007).

Many issues with regard to its ecology and population biology, however, remain unresolved. For example, the relationship between the populations of nematodes and density of *A. oligospora* under different field conditions needs to be established. On the one hand, the existence of nematodes can induce an increase in the number of *A. oligospora* propagules. On the other hand, the absence of nematodes did not significantly affect the population of this fungus. Furthermore, in some cases, the addition of *A. oligospora* did not significantly reduce the number of nematodes in field experiments. The underlying mechanisms governing population dynamics (Persmark et al., 1996) of *A. oligospora* are likely influenced by numerous factors, many of which remain relatively unexplored. For instance, its saprobic behavior in natural soil has not been quantified. From the perspective of fungal ecology, fundamental studies on its tritrophic (plant, nematode and nematode-trapping fungi) or multitrophic (plant, soil microorganisms, nematode and nematode-trapping fungi) interactions under natural conditions are required. Such complex interactions might have contributed to the inconsistent results among field experiments that incorporated *A. oligospora* as nematode control agents. To date, there has been no report of detrimental effects on the microbial community in the rhizosphere due to the applications of *A. oligospora*. It would be highly desirable if *A. oligospora* could trap and kill nematodes, but has no negative effect on beneficial microbes, such as mycorrhizae or nitrogen-fixing bacteria.

## The morphogenesis in *A. oligospora*

An association between morphogenesis and virulence has long been presumed for dimorphic fungi, typically with the saprobic stage being one morphotype and the infectious stage another type. *Arthrobotrys oligospora* can develop several different mycelial structures involved in predation, such as conidial traps, hyphal coils and the recently discovered appressoria, as well as three-dimensional sticky networks (Nordbring-Hertz, 2004). The typical adhesive network trap produced by *A. oligospora* (ATCC 24927)

consists of one to several loops attached to each other as a result of one to several anastomoses (Nordbring-Hertz, 1977b). An initial branch forms from a parental hypha that can be detected by its bright appearance under light microscope. The branch then curves around to meet a peg formed on the parent hypha some 20–25 μm from the initial branch to develop a loop. This loop typically consists of three cells, all with vigorous cytoplasmic movements. These cells differ from typical hyphal cells because they contain organelles called dense bodies and have the unique ability to capture nematodes (Figure 2) (Heintz and Pramer, 1972; Nordbring-Hertz, 1972; Nordbring-Hertz and Stalhammarcarlemalm, 1978). Electron microscopic observations showed that these organelles began to develop in the initial stages of trap formation (Veenhuis et al., 1984, 1985b). However, they were not present in vegetative cells, including those adjacent to the trap-initiating cell. The hyphal peg meeting the tip of the trap also lacked dense bodies, indicating that it was not a specialized trap cell up to the moment of contact and fusion. These dense bodies were cytosolic organelles which were peroxisomal in nature since they contained catalase and D-amino acid oxidase activity. These dense bodies were only detected in nematode-trapping fungi, but not in the so-called endoparasitic nematophagous fungi that infected their host with adhesive or non-adhesive spores (Veenhuis et al., 1985a). Their functions seemed to be involved in the adhesion of nematodes, but could be translocated into the developing trophic hypha after the nematode cuticle was penetrated. It has been assumed that they play a role in supplying energy and/or structural components to the invading hyphae (Veenhuis et al., 1989).

Another feature of trapping cells that makes them different from hyphal cells is the presence of extensive layers of extracellular polymers. These polymers have been considered important for the attachment of the traps to nematode surfaces (Tunlid et al., 1991). The extracellular fibrillar polymers consist of mainly proteins and carbohydrates. When the traps adhere to nematodes, they become denser and oriented toward one specific direction. Jensen and Lysek (1991) indicated that the attachment of hyphae of *A. oligospora* CBS 289.82 to second-stage juveniles of *M. hapla* was mediated by a layer of extracellular material produced by the fungus. The thickness of this extracellular material (about 0.1 μm) was comparable to similar layers found on other nematophagous fungi and less than the lumps of adhesive substances present on hyphaw of zygomycetes (Jensen and Lysek, 1991). Extracellular polymers, exclusively confined to trap cells were also isolated from both traps and vegetative hyphae in *A. oligospora* ATCC 24927 (Tunlid et al., 1991a). These polymers produced by *A. oligospora* ATCC 24927 were more loosely packed than the polymers in the layer bridging trap and nematode. They seemed to be distributed unevenly over the surface of *A. oligospora* ATCC 24927 (Belder et al., 1996). The structure of the adhesive layer could be quite complex and attachment could be accompanied by morphological changes (Nordbring-Hertz, 2004; Veenhuis et al., 1985b). It was found that the traps always showed a higher $K^+$ content than hyphae (Nordbring-Hertz et al., 1989). This data could be a result of higher metabolic activity in the trap cells than in hyphae. Moreover, the accumulation of $K^+$ might also be responsible for the pronounced turgidity of the traps (Veenhuis et al., 1985a).

Though the functions of adhesive networks were widely assumed to trap and kill nematodes, nematodes were not the only factor capable of inducing *A. oligospora* to form traps. In 1959, Pramer and Stoll (1959) provided unequivocal evidence that a metabolic product or a group of substances from the nematode *Neoaplectana glaseri*, collectively called "nemin", caused morphogenesis and induced trap formation in nematode-trapping fungi. However, the chemical nature of nemin has not been elucidated to date. Several experiments confirmed that when cultured in a

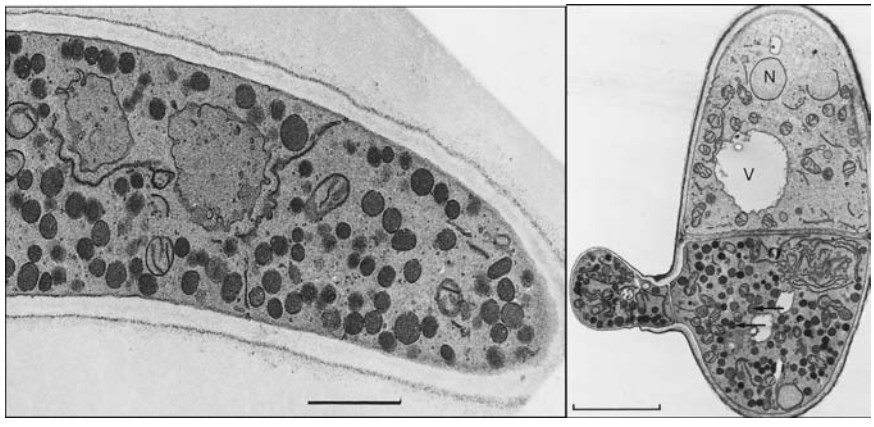


Figure 2. Transmission electron micrograph (left) of a trap cell of *A. oligospora* containing numerous typical dense bodies (Bar = 1 μm), and TEM micrograph (right) of germinating conidium with dense bodies both in CT and in the mother conidial cell (arrows), N: nucleus, V: vacuole (Bar = 5 μm) reproduced from Nordbring-Hertz (2004).

low nutrient medium, induction of trap formation could be brought about by adding small peptides or their constitutient amino acids (Friman et al., 1985; Lysek and Nordbringhertz, 1981). Many previous tests on the determining factors of the switch between pure saprophytism and predation under laboratory conditions indicated that trap formation could be stimulated by a low C:N ratio (Nguyen et al., 2007), i.e. by adding $NH_3$ at a certain concentration range (Jaffee and Strong, 2005), and by adding steroids, including lanosterol, ergosterol, phytosterol, $\beta$-sitosterol or cortisone acetate to cultures. In contrast, high concentrations of $CO_2$ at 5–10%, exposure to light and phosphate at concentrations above 30 M inhibited trap formation (Lee et al., 2004).

The capture of nematodes by *Arthrobotrys* spp. does not require a fully developed loop because nematodes can be trapped even by the initial branch of hypha. In an isolate of *A. superba*, nematodes were trapped by a basal cell, which later developed into either a full trap or into a conidiophore, depending on environmental conditions (Jansson and Nordbring-Hertz, 1981). This finding indicates that cells destined to become traps have the ability to trap nematodes long before the development of a full trap. In addition, growth conditions and environmental factors could strongly influence the direction of morphogenesis within this system (Werthmann-Cliemas and Lysek, 1986).

While the trap structures might be predominantly derived from mycelia, they could also form from spores directly upon germination without an intermediate hyphal phase. These structures, called conidial traps (CTs) (Nordbring-Hertz et al., 1995), were found in natural environments, such as cow dung (Dackman and Nordbring-Hertz, 1992) and rhizosphere soil (Persmark and Nordbring-Hertz, 1997). Conidial traps (Figures 1 and 2) contained numerous electron-dense bodies characteristic of normal hyphal network traps. They are capable of trapping nematodes as network traps. They adhere to a passing nematode and may be carried away and spread by the nematode in a way similar to adhesive conidia of endoparasitic nematophagous fungi. The production of conidial traps might indicate an increased potential of the fungus as antagonists to nematodes. Conidial traps have also been considered as survival structures, similar to conventional adhesive networks, based on the fact that adhesive net traps can survive long periods of time in the laboratory compared to normal hyphae (Dackman and Nordbring-Hertz, 1992). It is interesting that conidial traps of *A. oligospora* have never been detected in pure culture without the presence of natural substrates, such as dung or soil. When conidia of *A. oligospora* were incubated in the vicinity of cow faeces on agar plates, about 90% germinated into conidial traps. The occurrence of conidial traps in natural soil and soil extracts further supports the above-mentioned hypothesis. Studies on the mechanism of their formation showed that a low-nutrient medium was essential and that rhizosphere soil was more efficient than root-free soil for conidial traps formation. These observations have led to a proposal that the fungus competes for nutrients by forming conidial traps to overcome the fungistatic effects of the soil (Persmark and Nordbring-Hertz, 1997).

Another mechanism involved in the antagonism between *A. oligospora* and other fungi is the formation of hyphal coils around the hyphae of another fungus (Nordbring-Hertz, 2004). The properties of hyphal coils differ from those of vegetative hyphae, and are very similar to adhesive network traps. Coils also contain an abundance of cytoplasmic organelles that develop from the endoplasmic reticulum. However, dense bodies, typical of traps, are not present in coils. The function of hyphal coil is assumed to be associated with the mycoparasitic phase of *A. oligospora,* which, although a non-penetrating mycoparasite, is capable of deriving a considerable proportion of its nutrients from the host hyphae of other fungal species (Olsson and Persson, 1994).

Bordallo et al. (2002) reported that *A. oligospora* could colonize the surface of plant roots by forming appressoria during penetration of plant cell walls. However, this infection did not harm the development of the plants (Bordallo et al., 2002). The function of appressoria during plant infection by *A. oligospora* is still unclear.

Our recent studies of the effect of soil bacteria on the morphological diversity of *A. oligospora* (ATCC 24927) has shown that the fungus could produce a ring from mycelia at the initial stage in the vicinity of soil bacteria, which could further develop into a coil-like ring structure (unpublished work). Another interesting phenomenon, the formation of a 2D network, was also observed when *A. oligospora* was cultured without directly contacting nematodes. These observations provided a further example of the ability of this species to respond morphogenetically to environmental signals (unpublished work).

To date, five types of trapping devices – adhesive network, adhesive knob, adhesive column, nonconstricting ring and constricting ring – have been recognized and studied in predatory fungi. The first four trapping devices all contain an adhesive layer covering part or all of the device surfaces. The fifth and most sophisticated trapping device, the constricting ring, captures prey in a different way by swelling the three ring cells rapidly inwards and firmly lasso the victim within 1–2 s after being triggered by a nematode entering the ring. The morphogenesis and consequences of the high diversity of trapping devices among nematophagous fungi, as well as their value in biological control, have been described in several reviews (Barron, 1977, 1981; Dijksterhuis et al., 1994; Jansson and Lopez-Llorca, 2001; Kerry and Jaffee, 1997; Nordbring-Hertz et al., 2002). Different from other nematode-trapping fungi, many network-forming species do not form a network spontaneously. Their saprophytic state is more prominent and the formation of

network-trapping devices is induced by nematodes or nemin, a substance of animal origin (Pramer and Stoll, 1959). Spontaneous trap-producers are more effective at preying on nematodes than non-spontaneous trap formers because they have the flexibility to become more predacious by inducing more traps (Nordbring-Hertz et al., 2006). Recent attention has focused not only to adhesive nets, which are typical of the species involved in the infective process, but also to other hyphal structures that could contribute to their survival in the soil. Since *A. oligospora* is known as the most common network-forming species with a more competent saprophytic activity and quicker response to nematodes than other species, it has been regarded as the best model to study the relationship between morphology and functions of this special group of fungi. The morphogenesis of *A. oligospora* under different conditions has been studied intensively with the help of advanced microscopy.

On the basis of morphological features and/or molecular characters, various hypotheses on the evolution of trapping devices have been proposed. At present, the molecular mechanism of phenotypic switching in nematode-trapping fungi is not well understood. The main conflict among the hypotheses is the trapping structures themselves. Based on the observation that the adhesive network structure is the most widely distributed trapping device, Rubner (1996) suggested that it was the most advanced type of trapping organ. However, Li et al. (2000) considered that the adhesive trap was primitive due to its lower trapping efficiency. Based on phylogenies inferred from sequence analyses of 28S rDNA, 5.8S rDNA and $\beta$-tubulin genes, our recent study has indicated that the adhesive knob could be the ancestral type of trapping device from which constricting rings and networks were derived via two pathways (Li et al., 2005b). The deduction that the network-trapping device is one of the most evolved forms partly supported Rubner's theory. In a similar way, but with more comprehensive phylogenetic analysis of nucleotide sequences of three protein-coding genes (RNA polymerase II subunit gene, *rpb2*; elongation factor-1$\alpha$ gene, *ef-1α*; and $\beta$-tubulin gene, *bt*) and ribosomal DNA in the internal transcribed spacer region, Yang et al. (2007d) demonstrated that the adhesive network separated from the others early and represented an ancient type, supporting the hypothesis proposed by Li et al. (2005b) At present, the evolutionary origins and divergence of this network structure remained unresolved. Similarly, the molecular mechanism of phenotypic switching in *A. oligospora* and the genetic bases for the association between morphology and virulence are not well understood.

The conversion from vegetative mycelia to trapping devices in nematode-trapping fungi is crucial for pathogenesis. The ability of dimorphic pathogenic fungi to switch between different morphological states appears to be an important virulence determinant as mutant strains lacking this ability often have reduced virulence or are avirulent (Nemecek et al., 2006). Morphological switching is one aspect of the response to nutrient deprivation and, as such, is a response to an environmental stress. Pathogenic fungi appear to have adapted related cell signaling pathways to control morphological switching during infection. The genes controlling morphogenesis have, therefore, been the focus of many investigations, as they have great potential as targets for novel antifungal drugs (Nemecek et al., 2006). It has become clear that the cAMP signaling pathway is a major control mechanism for morphological switching in *Saccharomyces cerevisiae* and the signaling pathways in fungi are controlled by both cAMP and mitogen-activated protein kinase (MAPK) signal transduction pathways (Borges-Walmsley and Walmsley, 2000; Román et al., 2007). Epigenetic mechanisms may also be involved. For example, many epigenetic changes are controlled by the *SIR2* (silent information regulator) gene family (Brachmann et al., 1995). The *sir2*/*sir2* mutant of human fungal pathogen *Candida albicans* could undergo a much higher level of chromosomal alteration than wild-type strains and, therefore, exhibit a higher frequency of colony variants (José et al., 1999). To date, little is known about the control mechanism of morphological switching in *A. oligospora*.

### *A. oligospora* as a model organism for identifying nematocidal metabolites

In the 1950s, Shepherd (1955) observed that nematodes captured by fungi ceased to move, being either dead or paralyzed, before the bulb structure was completely developed. These observations led him to question Drechsler's assumption that the infection bulb produced by *A. oligospora* was the key factor that killed nematodes. In the 1960s, Olthof and Estey (1963) evaluated the effect of the filtrates of *A. oligospora* obtained from crushed nematodes on the vitality of nematodes in the genus *Rhabditis* sp., and found many worms were inactive and appeared dead. He concluded that the fungus could secrete a chemical substance which paralyzed or killed nematodes after they were caught by its adhesive trapping organs. This work provided a new perspective on the potential infective mechanisms that subsequently led to the discovery of a new group of nematophagous fungi that produced toxins to paralyze and kill nematodes. Over the past four decades, there has been a remarkable increase in the knowledge of the secondary metabolites of nematophagous fungi. The number of known substances from this special group of fungi with detrimental effects against nematodes has reached almost 200, and they are distributed among many classes, including alkaloids, peptides, terpenoids, macrolides, oxygen heterocycle and benzo compounds, quinones, aliphatic compounds, simple aromatic compounds, and sterols (Li et al., 2007a).

*A. oligospora* has a complement of secondary metabolites as numerous and diverse as those of other fungal taxa. Among the classes of compounds discovered in *A. oligospora* are polyketides, benzenoids and terpenoids. Additionally, other typical fungal secondary metabolites have also been observed in this species. These include large mixtures of compounds of several classes, such as lipids, peptides and sterols. Many secondary metabolites of *A. oligospora* appear to be associated with its nematicidal, antibacterial and antifungal properties. In the 1970s, Russian researchers showed that strains of *A. oligospora* produced wide-spectrum antibiotics against bacteria, actinomycetes and fungi (Kieu et al., 1971). Though several bioassays of strains of *A. oligospora* confirmed that this fungus could produce bioactive substances, the nature of these compounds was not elucidated until 1993. At that time, Stadler et al. (1993a) first reported that linoleic acid was the main nematicidal compound from several nematophagous fungi. In addition, the number of traps formed by *A. conoides* and *A. oligospora* was positively correlated to the concentration of linoleic acid and that this compound exhibited nematicidal activities towards the free-living nematode *Caenorhabditis elegans* with an $LD_{50}$ value of 5 g/ml.

Stadler et al. (1993b) also reported the isolation and structure elucidation of three new antibiotics with a novel carbon skeleton – oligosporon, oligosporol A and oligosporol B – from cultures of a strain of *A. oligospora* obtained from the Centraalbureau voor Schimmelcultures (Baarn, The Netherlands). Bioassays indicated that these compounds exhibited weak antimicrobial, cytotoxic and hemolytic effects, but were not active towards the nematode *C. elegans*. In addition, other *Arthrobotrys* species were also found to produce these or similar compounds. In the 1990s, three new derivatives of oligosporon, 4′,5′-dihydro-oligosporon, hydroxyoligosporon and 10′,11′-epoxyoligosporon, were obtained from an Australian isolate of *A. oligospora*, together with oligosporon and oligosporol B (Anderson et al., 1995). These oligosporon antibiotics shared a common structural feature: a farnesylated chain connected to an epoxy cyclohexen ring (Figure 3). The members of the oligosporon group displayed various biological activities from antibacterial and antifungal to nematocidal. For example, MIC values for oligosporon, oligosporol A and oligosporol B against the Gram-positive bacteria, *B. subtilis* and *S. aureofaciens,* were in the range 25–100 μg/ml Gram-negative and three Gram-positive bacteria. Dihydro- and oxidized analogues, 4′,5′-dihydro-oligosporon, hydroxyoligosporon and 10′,11′-epoxyoligosporon, were less active than oligosporon, oligosporol A or oligosporol B against the Gram-positive bacteria. In addition, oligosporon and 4′,5′-dihydro-oligosporon were found to inhibit vegetative growth of the plant pathogenic fungus *Phytophthora cinnamomi* Rands 1922 at <100 μg/ml, and retarded larval development of the intestinal parasitic nematode *Haemonchus contortus* with $LD_{50}$ values of 25 and 50–100 μg/ml. However, they were inactive against the nematode *C. elegans* at concentrations up to 100 μg/ml. The biogenesis of the oligosporon-type metabolites may be of mixed biosynthetic origins. The most plausible model for biosynthetic pathway leading to those metabolites was the condensation of a carbon skeleton formed by alkylation of a polyketide-derived cyclohexen nucleus with a terpenoid-derived farnesyl unit (Stadler et al., 1993b).

Secondary metabolites containing cyclohexen rings were widely distributed in fungi such as those in the genera *Eupenicillium*, *Phoma* and *Aspergilli*. However,

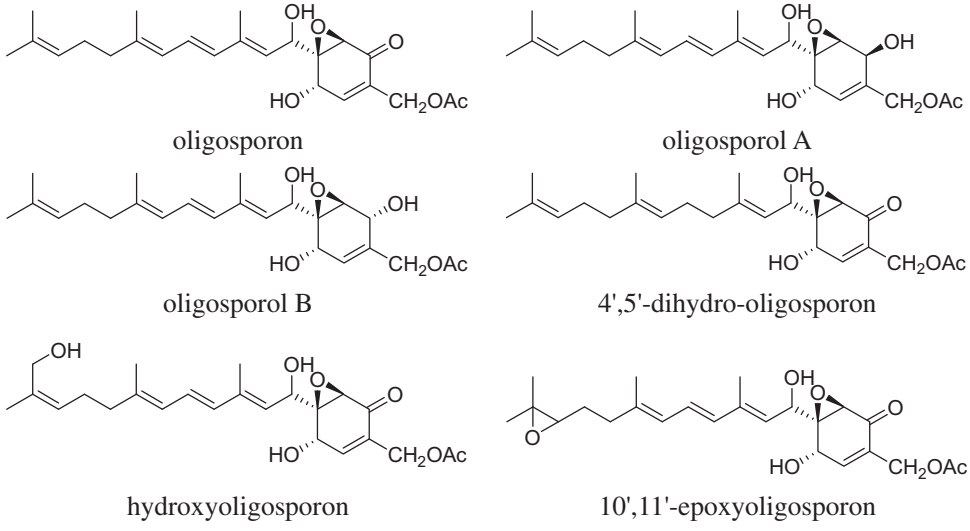


Figure 3. Unique oligosporon-type compounds from *A. oligospora.*

the compounds with a combination of an epoxy cyclohexen nucleus with a terpenoid-derived farnesyl unit were only found in nematophagous fungi (Li et al., 2007a). A nematode-trapping fungus, *Duddingtonia flagrans* Larsen 2000 was also reported to produce similar secondary metabolites, including a derivative of the oligosporon type, flagranones A, and two structurally related compounds, flagranones B and C, which possessed shorter chains attached to the cyclohexen rings (Anderson et al., 1999). These compounds showed similar antimicrobial activities. The oligosporon group represented the most complex structural type of nematocidal metabolites characterized so far from cultures of nematophagous fungi. They likely play a significant role in the interaction between nematophagous fungi and their nematode prey, and contribute to the potential ability of *A. oligospora* to protect crops and livestock from infestation by nematodes or microorganisms. Our recent studies on the metabolite profiles of the strains of *A. oligospora* from different environments revealed 10 new analogs of oligosporon and they differ from previously reported structures by lacking an acetyl group (unpublished work). In addition, the experiments suggested that the different strains of the species could yield oligosporon derivatives with different oxidation patterns. Both the novel structure and the biological activity of these oligosporon type metabolites warrant future investigations.

The tremendous developmental, structural and genetic variability of *A. oligospora* suggested that many more secondary metabolites likely remain to be discovered in this species. From the chemical point of view, this species will be an excellent model system for studying the function and evolution of nematophagous fungal secondary metabolism. Recent genome sequencing analyses revealed that the number of gene clusters presumably dedicated to secondary metabolism often exceeds the number of known compounds from a particular species. As is evident from the genomes of *Fusarium graminearum*, the *Aspergilli* and *Cochliobolus heterostrophus* (Drechsler) Drechsler 1934, the diversity of pathways in these fungi for the biosynthesis of natural products are much richer than expected (Hoffmeister and Kellerb, 2007). Similarly, we expect that *A. oligospora* has a large number of uncharacterized genes in gene families that are important for secondary metabolites. The availability of its genome sequences would lead to an enhanced effort in identifying biosynthetic genes for these molecules. Links between metabolism, light and sexual/asexual reproduction established in other fungi, such as *Aspergillus* spp. and *Fusarium* spp., are allowing researchers to explore novel metabolites and their biological function by using genomic approaches (Hoffmeister and Kellerb, 2007). The near future will witness how whole metabolomes of a species or even a genus are explored and complemented by investigations into their ecological roles. However, the roles of most secondary metabolites in fungi remain largely unknown. Many of these fungi live saprophytically in the soil and such molecules may provide protection against competitors and/or predators in this ecological niche. Understanding the key factors affecting the dynamics of *A. oligospora* could lead to improvements in its deployment as a biocontrol agent. Similarly, understanding the molecular interactions during infections may lead to the identification of new targets and the discovery of new bioactive compounds. *A. oligospora*, a fungus with unusual combination of saprophytism and parasitism, could serve as an excellent model for exploring the link between secondary metabolism and biological function.

## *A. oligospora* as a model organism to study lectins that target nematode receptors

Fungi are heterotrophic organisms that depend on saprophitism, symbiosis or parasitism for their sources of carbon and energy. These lifestyles require specific recognition between the fungus and the organic matter or host tissue for adhesion and subsequent invasion. The existence of specialized fungal proteins capable of binding to sugar and other substances suggests that fungi have developed a strategy to bind to host glycoconjugates by producing a type of protein called lectins, which target specific tissues (Sharon and Lis, 1972). Lectins include a diverse group of carbohydrate-binding proteins commonly present in animals, plants and microorganisms (Sharon and Lis-Sharon, 1989; Wimmerova et al., 2003).

As for nematode-trapping fungi, recognition and adhesion were the first steps in the infection of prey. One of the first examples indicating a lectin-mediated interaction in a fungal–host system involved the nematode-trapping fungus *A. oligospora*. In the late 1970s, Nordbring-Hertz and Mattiasson (1979) observed that the nematode-capturing ability of *A. oligospora* was inhibited by various sugars and suggested that entrapment was mediated by the interaction between a lectin on the surface of the fungal trap and a specific sugar on the nematode cuticle. Prey recognition by the fungus has been attributed to a molecular interaction of certain proteins on the fungal surface with sugar molecules on the nematode cuticle. Application of affinity chromatography led to the isolation of a GalNAc-specific protein from homogenates from *A. oligospora* mycelium, which displayed binding characteristics typical of lectins. In 1984, the molecular weight of the protein was estimated at 22,000 Da based on its mobility on SDS–polyacrylamide slab gels (Borrebaeck et al., 1984). Pretreatment of nematodes with the purified protein reduced entrapment. The presence of GalNAc residues on the nematode cuticle suggested that the protein might have a role in the recognition and capture of nematodes by the fungus. Results from inhibition experiments using various soluble carbohydrates supported that the adhesion was initiated by a GalNAc-specific lectin in the fungus binding to a carbohydrate receptor present on the nematode surface (Premachandran

and Pramer, 1984). Several similar experiments have indicated that lectins were involved in the adhesion to host surfaces in both parasitic and symbiotic fungi (Premachandran and Pramer, 1984). In 1992, experiments with the application of mucin–Sepharose columns resulted in the isolation of a lectin (designated AOL) from *A. oligospora* (Rosén et al., 1992). The lectin had a similar molecular mass and antigenicity as a previously isolated Gal-NAc-specific lectin. This new lectin was a saline-soluble, hemagglutinating protein that consisted of two identical, non-covalently associated subunits (16 kDa). Evidence showed that AOL was a multispecific protein that bound not only to ligands containing GalNAc residues (present in glycoproteins in the sequence GalP3GalNAca-Ser/Thr) but also to sulfated glycoconjugates (e.g. sulfatide and fucoidan) and to two phospholipids. The binding specificity to GalP3GalNAccx-SerlThr was similar to that identified for a lectin ABL isolated from the mushroom *Agaricus bisporus* (Rosén et al., 1996a). Further assays demonstrated that AOL could interact with several other glycoproteins containing O-linked and/or N-linked sugar chains. In 1996, the gene encoding the lectin AOL from *A. oligospora* was cloned and analyzed (Rosén et al., 1996b). The deduced primary structure of the AOL gene had a high sequence similarity (identity 46.3%) to the deduced amino acid sequence of the cDNA clone of ABL, but not to any other fungal, plant or animal lectins, which confirmed that AOL and ABL were members of the same family of saline-soluble lectins present in fungi sharing similar primary structures and binding properties (Rosén et al., 1997). Electrospray mass spectrometric analysis indicated that AOL had an acetylated N-terminal but no other posttranslational modifications. Circular dichroism (CD) spectroscopy suggested that the secondary structure of AOL contained 34% P-sheets, 21 % u-helix, and 45% turns and coils (Rosén et al., 1997). Since the protein was found to be present in the cytoplasm and not at the surface of the trap cells, it has been proposed that AOL can function as a storage protein during saprophytic and pathogenetic growth (Rosén et al., 1997).

In the early 2000, cloning and recombination techniques were applied to study the functions of AOL (Åhman et al., 2002). A gene encoding the lectin (AOL) was deleted in *A. oligospora*, and the mutant showed no agglutination activity or no cross-reacting with AOL antibodies (Balogh et al., 2003). However, no significant difference between the mutant and wild-type strains in spore (conidia) germination, saprophytic growth and pathogenicity was observed. Furthermore, there was no significant difference in the growth and reproduction of collembolan feeding on the various strains of *A. oligospora* (Balogh et al., 2003). The findings confirmed an earlier hypothesis that AOL was not the only factor involved in mediating the interaction between the nematode and the fungus (Åhman et al., 2002). The possible explanation is that AOL is a component of a system of defense against various animal fungivores, or that the fungus can compensate for the absence of the lectin by expressing other proteins with similar function(s) as AOL.

Lectins have been isolated from more than 60 fungi including saprophytic, parasitic, and symbiotic species. Studies of fungal lectins have been mostly focused on mushrooms (Wang et al., 1998). Many of them are saline-soluble proteins consisting of one or several low-molecular-weight subunits. A large number of mushroom lectins have now been sequenced and characterized, and interest arose when clear similarity with human galectins and immunoglobulins was established. It has been proposed that they are involved in storage of nutrients, development, recognition of other organisms and defense reactions (Wimmerova et al., 2003). Among them, the lectin ABL identified from the mushroom *Agaricus bisporus* was found to be similar to the lectin AOL isolated from *A. oligospora*. As for nematode-trapping fungi, if their predatory activity was directed by reactions with the specificity of lectins, the fungi would be expected to be selective in their choice of prey. Nevertheless, that is generally not the case; most trapping fungi are indiscriminatory with regard to their prey nematodes. A single fungal species can trap many different species of nematodes and even other animals of microscopic dimensions. Nematode-trapping fungi may depend on lectin for prey recognition, but it is doubtful that lectins can account fully for the broad spectrum of activity and the remarkable tenacity of the mucilage produced by *A. oligospora* and other species. However, the above explanation does not exclude the possibility that *A. oligospora* produces a lectin that specifically recognizes sugar residues common to many or all nematodes (Wimmerova et al., 2003).

### *A. oligospora* as a model organism to identify proteases that target nematode cuticles

The nematode cuticle contains a solid exoskeleton composed mainly of proteins that act as a barrier against environment stresses and potential pathogen attacks (Cox et al., 1981). The mechanism by which nematode trapping fungi penetrate the surface of their prey has not been fully elucidated. Current consensus is that the invasion involves enzymatic actions. This is because extracellular proteases have been implicated in the penetration and digestion of host tissues by many plant and animal pathogenic fungi, and there is increasing evidence from ultrastructural and histochemical studies showing that extracellular hydrolytic enzymes such as proteases, collagenase and chitinase are involved in nematode-cuticle penetration and host-cell digestion (Yang et al., 2007a).

Since most studies on the chemical composition of nematode cuticles have revealed collagen as the main component of the nematode cuticle, collagenases from predatory fungi have been assumed to play a key role in

infection against nematodes (Blaxter and Robertson, 1998; Huang et al., 2004)). Collagens are among the most complex proteins and degrade slowly in natural soils and waters (Blaxter and Robertson, 1998). Schenck et al. (1980) examined seven *Arthrobotrys* species and found that these fungi produced collagenases when they were grown in liquid medium free of proteosepeptone (proteosepeptone induces collagenase production). Tosi et al. (2001) observed that the production of collagenases in an Antarctic strain of *Arthrobotrys tortor* Jarowaja was threefold higher than other species of the *Arthrobotrys* genus. However, to date, these are the only reports on collagenase production by nematophagous fungi.

In the early of 1990s, Tunlid and Jansson (1991) showed that *A. oligospora* produced extracellular proteases during its infection of nematodes. These proteases were very sensitive to inhibitors such as phenylmethyl sulfony fluoride (PMSF), chymostatin and antipain. These results indicated that the proteases belong to serine proteases. Serine proteases are a family of enzymes that utilize a uniquely activated serine residue in the substrate-binding pocket to catalytically hydrolyze peptide bonds (Schultz and Liebman, 1997). Bioassays performed with various inhibitors showed that the activity of proteases from *A. oligospora* was not involved in the adhesion of nematodes to the traps. Incubating the trap-bearing mycelium with inhibitors against serine proteases significantly decreased the immobilization of captured nematodes, indicative of an important function of such proteases during the infection of nematodes (Tunlid and Jansson, 1991). The production of proteases could be stimulated by nematode cuticle. Further studies led to the purification and characterization of an extracellular serine protease (PII) in the culture filtrates of *A. oligospora* (Tunlid et al., 1994). This extracellular serine protease was capable of hydrolyzing cuticle proteins and immobilizing free-living nematodes, which suggested that it is likely an important virulent factor for the infection of nematodes by *A. oligospora*. These studies provided the first insights into the molecular mechanism by which nematode-trapping fungi penetrated the surface of their prey. Åhman et al. (1996) revealed that the primary sequence of a gene encoding PII showed a high degree of similarity with members of the subtilisin family of ascomycetes. Northern blotting analysis demonstrated that *PII* was expressed when the fungus was starved of nitrogen and carbon. In addition, the expression of *PII* was significantly stimulated by the addition of various proteins including fragments of nematode cuticle. The transcript of *PII* was not detected during the early stages of infection (adhesion and penetration), but high levels were detected concurrent with the killing and colonization of the nematodes.

In the 2000s, several *PII* mutants were generated by Åhman et al. (2002) through targeted gene knockout to investigate the role of PII in *A. oligospora*. Mutants containing additional copies of the *PII* gene developed a higher number of infection structures and had an increased speed of capturing and killing nematodes than the wild-type strain. This result suggested that genetic manipulation could be used to improve the virulence of a nematode-trapping fungus. The recombinant enzyme coming from PII expressed in a heterologous system (*A. niger*) also showed nematotoxic activity in vitro when added to free-living nematodes. Disruption of the *PII* gene by homologous recombination had a limited effect on the pathogenicity of the fungus. The toxic activity of PII was significantly higher than that of other commercially available serine proteases.

In 2004, our group characterized a homolog of *PII*, designated *Aoz1*, in accordance with gene nomenclature in other fungi, and its protein (Aoz1) from an isolate of *A. oligospora* from Yunnan Province (Zhao et al., 2004). The expression of this neutral serine protease was enhanced by the addition of gelatin to the culture medium. In addition, this protease immobilized nematodes and degraded nematode cuticles. Based on BLASTP analysis, the deduced primary sequence of Aoz1 showed extensive similarity with proteases of the subtilase family of serine endopeptidases, including the conservation of serine, histidine and aspartate components of the active site in subtilisins. The apparent homologies suggested that *A. oligospora* might contain multiple related proteases. In addition, PCR products derived from our degenerate primer pool revealed three DNA bands on agarose gels (900, 1.2 and 1.5 kb), only one of which was characterized in that study (Zhao et al., 2004). Åhman et al (1996) also reported that Southern-blot analysis of genomic DNA of *A. oligospora* performed under moderate stringency resulted in several minor bands in addition to that corresponding to *PII.* Our report provided support for the important role of one or more proteases in the pathogenicity of *A. oligospora* toward nematodes. The characterization of the second enzyme and its gene provided direct evidence that the fungus could produce a series of functionally and structurally related extracellular serine proteases during the infection process. These studies also established the foundations for future investigations into the structure–function relationships of cuticle degrading proteases, and for improving the pathogenicity of nematophagous fungi and possibly for engineering crop resistance against nematodes. Studies have indicated that nematophagous fungi could compensate for their loss of proteolytic activity by expressing other hydrolytic enzymes. From an evolutionary perspective, this might be a useful strategy since many hosts, including plants, insects and nematodes, are known to be capable of producing serine protease inhibitors.

Since we first found an extracellular serine protease Aoz1 from *A. oligospora* in 2004, several extracellular serine proteases have been identified from other nematophagous fungi by our group. These included Ac1

from *Arthrobotrys conoides* (Yang et al., 2007b), Ds1 from *Dactylella shizishanna* (Wang et al., 2006b), Dv1 from *D. varietas* (Yang et al., 2007c), Mlx from *Monacrosporium. microscaphoides* (Wang et al., 2006a), Mc1 from *M. cystosporium* (Yang et al., 2008), PrC from *Clonostachys rosea* (Li et al., 2006) and Ver112 from *Lecanicillium psalliotae* (Yang et al., 2005). In total, our group has contributed to half of the extracellular serine proteases from nematophagous fungi to date in public databases. The biochemical properties of proteases isolated from nematode trapping fungi are all very similar. They have similar molecular weights ranging from 32 to 39 kDa and share a broad range of protein substrates including casein, gelatin, nematode cuticle, eggshells, etc. Sequence analyses and comparisons showed that serine proteases from nematophagous fungi shared extensive similarities to the subtilisin family of serine proteases from non-nematophagous fungi, all possessing a pre-pro-peptide structure (Gunkel and Gassen, 1998). The PII from *A. oligospora* showed 88.2, 84.7, 84.4, 66.4, 41.3, 43.5, 39, 40, 41.7 and 38.8% identity, respectively, to Ac1, Mlx, Mc1, Dv1, pSP-3 from *Paecilomyces lilacinus* (Bonants et al., 1995), VCP1 from *Verticillium chlamydosporia* (Morton et al., 2003), Ver112, prot K from *Tritirachium album*, Pr1 from *Beauveria bassiana* (Joshi et al., 1995), and PrA (*Metarhizium anisopliae*) (St Leger et al., 1992). According to the phylogenetic tree (Figure 4) constructed on the basis of the deduced amino acid sequences from nematophagous and entomopathogenic fungi by the Mega program package (Tamura et al., 2007), six proteases (Mc1, Dv1, PII, Aoz1, Ac1, and Mlx) identified from nematode-trapping fungi formed a clade. The clustering of nematode-trapping fungi was consistent with their taxonomic affiliations. Based on the phylogenetic analyses, Yang et al. (2008) proposed that the pathogenicity related serine proteases from nematophagous and entomopathogenic fungi have evolved from a common ancestor.

Recently, our group reported the crystal structures of the two proteases, Ver112 from *L. psalliotae* and PL646 from *Paecilomyces lilacinus* (syn. pSP-3 from *P. lilacinus*) (Liang et al., 2010). Both Ver112 and PL646 showed high hydrolytic activities against cuticle proteins derived from *C. elegans* and other substrates at broad ranges of temperatures and pH. The crystal structures of PL646 and Ver112 were very similar, and both consisted of six helices, a nine-stranded parallel sheet and three two-stranded antiparallel sheets. Differences between the structures were found among residues of the substrate binding sites (S1 and S4). The substrate-binding pockets within both enzymes are large and in the case of S1, hydrophobic. The electrostatic surface potentials of the two proteases demonstrated that they have a common feature: only the surfaces on the substrate-binding regions were negatively charged, while the remaining surfaces of the molecules positively charged. The anionic substrate-binding regions could increase the local conformational flexibility and enhance catalytic efficiency (Baker et al., 2001), and the large positively charged areas on most of the molecular surface could increase the adsorption of the cuticle-degrading proteases to cuticles bearing abundant acidic residues. The electrostatic surface features of these cuticle-degrading proteases likely contribute significantly to fungal infection against nematodes. In addition, both the structures of PL646 and Ver112 were similar to that of proteinase K from *T. album* (Liang et al., 2010). This observation suggested that proteinase K, Ver112 and PL646, though produced by different fungal species, work by similar mechanisms during fungal infections (Schultz and Liebman, 1997).

An increasing number of studies suggested that serine proteases are significant pathogenic factors found in bacterial or fungal pathogens against insects, nematodes and even humans (Yang et al., 2007a). To date, *A. oligospora* is the only carnivorous fungus with two extracellular serine proteases involved in the infective process. Further studies on its extracellular proteases will help reveal the roles of

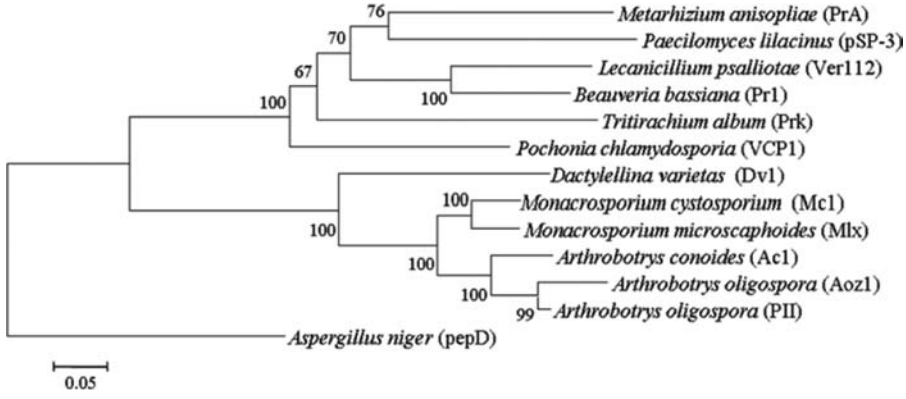


Figure 4. Phylogenetic tree showing the relationship of fungal serine proteases, reproduced from Yang et al. (2008).

these enzymes and their potential synergies, and contribute to further understanding of the infective mechanism of this type of agriculturally important beneficial fungi.

## Biodiversity of nematophagous fungi

Initial interests in studying the interaction between fungi and their nematode hosts were focused on their potential as biological control agents of plant parasitic nematodes. These nematode pests are among the most destructive groups of plant pathogens worldwide and are extremely challenging to control (Chandrawathani et al., 1998; Moens and Perry, 2009). Though the fungal kingdom comprises about 1.5 million species (Hawksworth, 2001), only about 160 species of fungi (distributed in Zygomycota, Basidiomycota and Ascomycota) are known capable of capturing nematodes (Li et al., 2000). Nematophagous fungi have been studied for their nematode-killing capabilities, ecology and their general biology. Several reviews on various aspects of this group of fungi have been published (Barron, 1977, 1981; Dijksterhuis et al., 1994; Grønvold et al., 1993a; Jansson and Lopez-Llorca, 2001; Kerry and Jaffee, 1997; Liu et al., 2009; Nordbring-Hertz et al., 2002). Most known nematophagous fungi could be classified into four major classes according to their infective strategies: trapping, endoparasitic, opportunistic and toxic fungi.

Nematode trapping fungi are the most common predatory fungi. They have a wide range of suppressive activities on different nematode species, including free-living and predatory nematodes as well as animal- and plant-parasitic nematodes. Because nitrogen is essential for fungal growth and not freely available either in dead wood or in soil where carbon is abundant, direct capture of other living life forms for nitrogen compounds would be an advantage (Barron, 2003). Nematode-trapping fungi have evolved predatory organs and often have increased capacity for predation under low-nutrient environment (Borges-Walmsley and Walmsley, 2000). They can form different trapping devices derived from hyphae to infect nematodes. Several trapping devices have been recognized, including three-dimensional adhesive network, two-dimensional adhesive network, adhesive hyphae, adhesive knobs, adhesive branches, constricting rings, and non-constricting rings (Figure 5). The ultrastructures of these nematode-trapping devices have been extensively studied (Li et al., 2000). It is noteworthy that adhesive traps (branches, nets, hyphae and knobs), though varying in morphology, share some common features such as containing numerous cytosolic organelles (e.g. dense bodies) within the trapping hyphal cells and extensive layers of extracellular polymers covering the traps (Tunlid et al., 1991). Trapping structures are specialized tools that nematode-trapping fungi use for obtaining a broad range of food supplies. These traps are all derived from sparse mycelia to capture and infect nematodes. During the infection process, the prey cuticle is penetrated, the nematode immobilized, and the

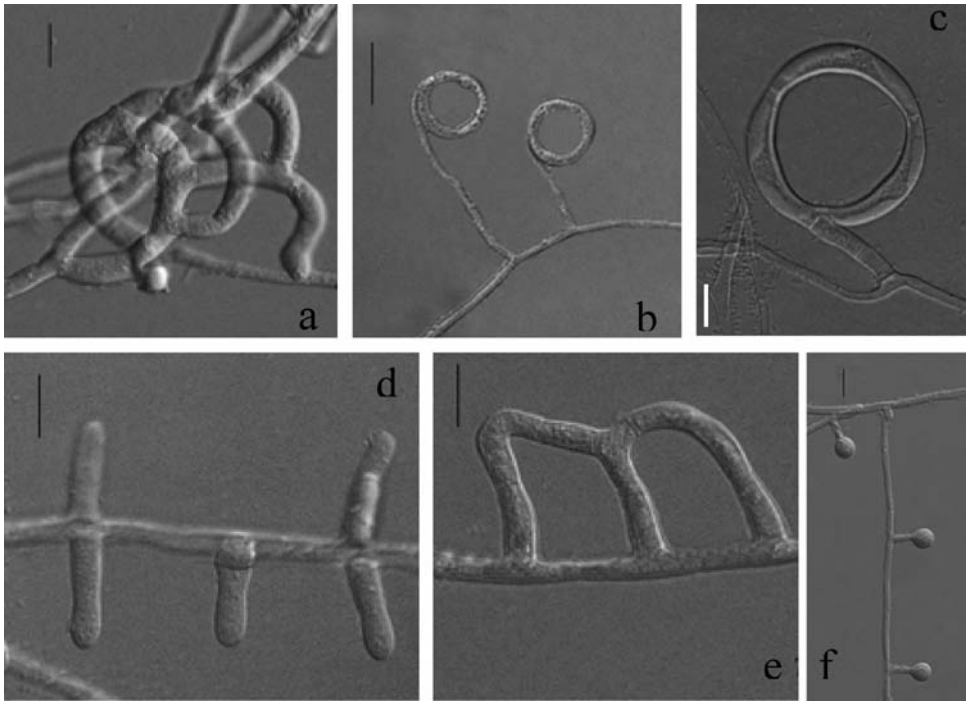


Figure 5. Diversity of trapping structures of nematophagous fungi. (a) Adhesive network; (b) non-constricting rings; (c) constricting ring; (d) adhesive branches; (e) adhesive 2D net; (f) adhesive knob (bar = 10 μm), Reproduced from Yang et al. (2007a) and Zhang and Mo (2006).

prey eventually invaded and digested by the fungus (Liu et al., 2009). Despite this remarkable biological adaptation, these fungi are not obligate predators (Onofri et al., 2007). In pure culture or in a nematode-free environment, they grow as saprophytes. However, if nematodes are present, hyphae differentiate to form trap structures. Hence, the fungal predatory structure is contingent upon contact with a nematode. From morphological and functional points of view, trapping devices are more informative than asexual reproductive structures for grouping the nematode-trapping fungi (Yang et al., 2007d).

In contrast to nematode-trapping fungi, endoparasitic fungi are often obligate parasites and have no or only a limited saprophytic phase. They produce almost no mycelium in soil and their whole lifecycles occur within the body of their hosts. The endoparasites of nematodes show considerable diversity with encysting zoospores belonging to the Chytridiomycetes and Oomycetes (Bordallo et al., 2002; Li et al., 2000; Persmark et al., 1992). Endoparasites initiate the infection process with adhesive spores when conidia adhere to the nematode cuticle. The nature of the adhesive spores differs between genera. The conidia of some species have been observed to be capable of attracting nematodes (Jansson, 1982). However, due to their limited growth in culture, their poor competitive saprophytic ability and the susceptibility of their spores to mycostasis, it might be very difficult for them to get established in a new environment. Therefore, it seems that these fungi might be of relatively limited use in biocontrol applications.

The group of fungi that usually live as saprophytes but can use nematodes as one of their nutrient resources are often described as opportunistic nematophagous fungi (Jansson and Lopez-Llorca, 2001). This group is represented by *Paecilomyces lilacinus* and *Pochonia chlamydosporium* (*Verticillium chlamydosporium*) (Khan et al., 2004; Lopez-Llorca et al., 2002). Nematodes belonging to the *Heteroderid* group and at the sedentary stages of their lifecycles are vulnerable to attack by these fungi. Such attacks could happen within the host plant roots, on the root surface, or in the soil away from roots. These fungi can colonize nematode reproductive structures, penetrating the cuticle barrier to infect and kill the nematode hosts. Once in contact with cysts or egg masses of nematodes, these fungi also grow rapidly and eventually parasitize all eggs that are in the early embryonic stages of development. Though they cannot form trapping devices, scanning electron microscopic observations revealed that *P. chlasporium* could produce appressoria on the host surface and accumulate a mucilaginous material between the appressoria and the eggshell (Lopez-Llorca et al., 2002). This material could function as an adhesin to assist eggshell penetration by the fungus. As with nematode-trapping fungi, opportunistic nematophagous fungi also use extracellular hydrolytic enzymes to penetrate their hosts.

There s also a group of fungi which can produce nematicidal toxins to attack nematodes. With the reduced use of synthetic chemical nematicides and increased demands for environmentally friendly alternatives in recent years, searching for natural nematicidal toxins from fungi for the management of nematode pests has attracted increasing attention. Significant research in this area in the recent past has led to the discovery of more than 200 structurally diverse nematicidal compounds from about 60 fungi (Li et al., 2007a). Though no major commercial product based on these natural fungal compounds has been developed, several candidate compounds are under intense research and development.

In addition to the above four groups of nematophagous fungi, a novel mode of action of fungi against nematodes was found by our group (Luo et al., 2004, 2004). Two species belonging to basidiomycetous fungi, *Stropharia rugosoannulata* and *Coprinus comatus* (Figure 6) were observed to produce a special nematode-attacking device: an acanthocyte. Microscopic observations showed that some acanthae resembled a sharp sword that could cause damage to the nematode cuticle, resulting in leakage of nematode inner materials. This result suggested that mechanical force could be a very important virulence factor in these fungi (Luo et al., 2006).

With the availability of new tools to investigate complex microbial communities at specific sites in the environment and the expanded appreciation for the importance of the nematophagous fungi, it is an opportune time to apply modern ecological and evolutionary principles to improve our current understanding of nematophagous fungi. For example, the use of genome sequences and related approaches (such as whole-community fingerprinting methods) (Fuhrman, 2009; Giovannoni and Stingl, 2005) could overcome the need for cultivation to allow us directly characterize and identify nematophagous microorganisms in nature.

It is noteworthy that, compared with the other three types of nematophagous fungi, nematode-trapping fungi share a unique ability to form specialized morphological structures – traps to capture nematode (Li et al., 2000). In nature, nematode-trapping fungi are likely to be more abundant and more diverse than the other three types of nematophagous fungi. Their abundance may be due to their greater competitiveness and/or their superior dispersal ability (Fuhrman, 2009). These common organisms are very important for nutrient cycling in the ecosystem (Fuhrman, 2009). On the applied side, more attention is being given to the selection of broad-spectrum nematode-destroying fungi and improvements in the production, formulation and application technologies for their use in controlling pest nematodes. Efforts are also being made to optimize the impact of these fungi by integrating them with other novel crop protection strategies. Increasing evidence suggests that *A. oligospora* is by far the most common

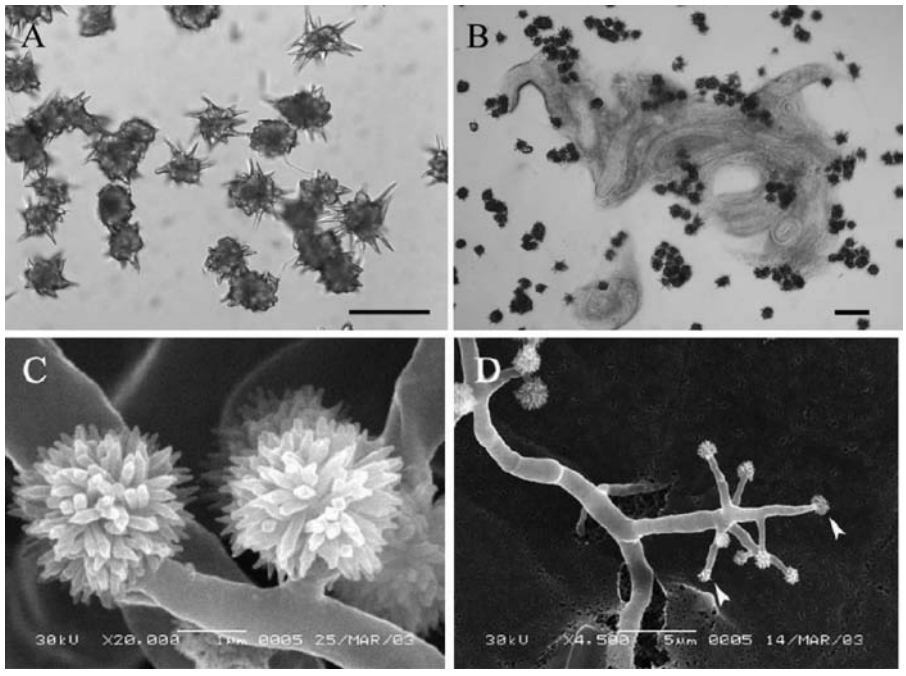


Figure 6. (A) Acanthocytes of *Stropharia rugosoannulata* (bar = 20 μm), reproduced from Luo et al. (2006). (B) Nematodes immobilized by purified acanthocytes of *Stropharia rugosoannulata* 12 h after addition of the nematodes (bar = 100 μm). (C, D) Spinyballs of *Coprinus comatus*. Reproduced from Luo et al. (2004).

species among the members of nematode-trapping fungi. It has an active saprophytic stage and responds well to nematodes by producing adhesive network, allowing it to switch quickly from a saprophyte to predatory lifestyle. Current evidence suggests that *A. oligospora* is among the biggest contributors to the population of nematodes. These attributes make *A. oligospora* a model organism for analyzing the characteristics that render saprophytic microorganisms pathogenic under certain environmental circumstances.

## The potential significance of nematophagous fungi as biological control agents

The phylum Nematoda includes parasites of plants and animals. They are among the most abundant multicellular animals on earth. Numerically, between 80 and 90% of all multicellular animals on earth may be nematodes (Bloemers et al., 1997; Jairajpuri and Åhmad, 1992). It has been long known that parasitic nematodes cause numerous diseases in humans, animals and plants, and these parasitic nematodes have long been recognized as a major contributor to the decreasing quantity and quality of agricultural products and livestock.

Phytoparasitic nematodes are among the most notoriously difficult crop pests to control. Depending on the crop field, plant nematodes can cause complete crop failure (Mitreva et al., 2007). Historically, the control and management of nematode-induced crop damages are achieved through breeding resistant plants, crop rotation and other cultural practices, and/or chemical nematicides. The suppression of nematodes in the past has been primarily achieved through the widespread use of in-furrow organophosphate and carbamate insecticides. As the concern for potential health and environmental effects of these agrochemicals increases, there is an urgent need for biocontrol measures. In addition, there is evidence that nematodes are adapting to chemical nematocides and such nematodes are causing increasingly more damages to crops (Mitreva et al., 2007). Recently, researchers at a Scottish agricultural college showed that free-living nematodes caused greater loss in potato crops at lower population numbers, given the previous assumption that growers need not worry about free living nematodes, particularly with populations smaller than 100 nematodes in 250 g of soil. The new findings have also indicated that the numbers of nematodes had increased by 300% over the past decade in many crop fields (Dieterich and Sommer, 2009). The need for greater sustainability in agriculture and for improving crop yield to help solve the looming world food crisis has led researchers to pay more attention to biological control for its environmentally friendly outcomes.

Furthermore, soil-transmitted nematode (STN) infections represent a major cause of morbidity in developing countries, with an estimated burden of human disease, comparable with that of malaria or tuberculosis. In addition to human health, animal nematode infections are of major veterinary significance, resulting in millions of dollars of

lost revenue for industries that provide products and food from livestock, including cattle and sheep. Although anti-nematode drugs (anthelminthics) exist, there are increasing concerns about the emergence of resistance to these compounds. Hence, there exists a pressing need to develop new, safe and inexpensive agents for the treatment of human and veterinary nematode infections (Dieterich and Sommer, 2009).

The use of beneficial microorganisms (biopesticides) has been considered one of the most promising methods for more rational and safe parasitic nematode-control practices (McSorley et al., 2008). Of the microorganisms that parasitize or prey on nematodes, fungi are estimated to contribute up to 80% of the total microbial biomass in many soils and hold an important position in continuously destroying nematodes in virtually all types of soils. The fungal antagonism consists of a great variety of organisms which vary considerably in their biology and taxonomy and play a major role in recycling carbon, nitrogen and other important elements from the rather substantial biomass of nematodes (McSorley et al., 2008; Nordbring-Hertz et al., 2002). Some 70 genera and 200 species of fungi have been found associated with nematodes. To date, only a few are successful biocontrol agents. It will take a considerable amount of time before the potential of many of these fungi as biocontrol agents is realized. Successful control relies on having a sufficiently high density of the fungus to be maintained in the natural environment. The requirement for high densities of agents to control the pest applies across all biocontrol efforts. One of the challenges is to develop methods to produce and apply high densities of fungi under practical farming conditions (Li et al., 2000; Nordbring-Hertz et al., 2000).

The genome sequencing of *A. oligospora* strain ATCC 24927 was recently completed. The availability of a complete genome sequence of this fungus will pave the way for understanding the genetic background of the specialized predaceous structure and virulence determinants of this microorganism. Additional information regarding differences between nematode-trapping and non-parasitic species will provide insights into the evolution and, potentially, the nature of parasitism. Genetic and genomic approaches to study nematode-trapping fungi now have a solid foundation. For example, our most recent investigation revealed that a knockout mutant of *A. oligospora* (ATCC 24927) constructed with the help of the genome annotation showed much more nematocidal activity than its wild type (unpublished work).

Systems biology approaches are becoming increasingly helpful to unravel, predict and quantify nematode-killing abilities within particular organisms or microbial consortia in individual niches. Approaches to predict and quantify the predaceous capabilities of particular organisms or microbial consortia have long before appeared, but a combination of such approaches with mechanistic knowledge of pathogenesis processes, the elucidation of structure–function relationships and the knowledge on the ecology of this microorganism will provide the basis for successful regulation of nematode populations, leading to improved biocontrol strategies and methods. In addition, the ability of *A. oligospora* to adapt to a diversity of ecosystems may be associated with certain genomic signatures. Large-scale genomic, transcriptomic and proteomic studies can provide a unique entry point into interdisciplinary investigations of *A. oligospora* parasitism and an ecological and evolutionary perspective on fungi–nematode coevolution.

Meanwhile, we suggest that the complex interaction between soil bacteria and nematode-trapping fungi might have contributed to the evolution and maintenance of virulence factors and their associated genes (Kobayashi and Crouch, 2009). Recent studies revealed that soil bacteria not only produced fungistatic compounds (Chuankun et al., 2004) but also induced fungal morphological changes in the soil. Our group observed that the culture filtrate of a *Bacillus* strain H6, representative of the dominant colony types isolated from fungistatic soils, could induce unusual swelling in the conidia and the germ tubes of nematophagous fungi, and prevent the fungi from proliferation (Li et al., 2005a, 2007b). Further study displayed that another *Bacillus* strain could induce *A. oligospora* to form two-dimensional networks and cosla-like coil rings. Hence, investigations into bacteria–fungi interactions could provide insights into microbial ecology that might also extend to mechanisms of pathogenesis. *A. oligospora* is potentially an excellent model for studying the evolution of nematode-trapping fungi in soil and is an important gene pool for future agricultural genetic engineering prospects. Along these lines, the *A. oligospora–Pseudomonas* and *A. oligospora–Bacillus* interactions may provide insight into the microbial ecology of bacteria and fungi in ecological niches outside vertebrate hosts.

## Summary

*A. oligospora* is an opportunistic nematode pathogen discovered more than a century ago, and is the most extensively studied nematode-destroying fungal species. The salient feature of this species is that it forms three-dimensional network traps that capture nematodes (Hashmi and Connan, 1989). Improvements have been made through the discovery of pathogenic factors, or by creating new ways of presenting the factors to the target nematodes. A detailed understanding of how *A. oligospora* interacts with its host should facilitate the design of more effective biological control products. The results obtained using *A. oligospora* will stimulate new approaches to solving long-standing problems in fungal–nematode interactions and pest nematode controls.

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## References

Åhman J, Ek B, Rask L, Tunlid A. 1996. Sequence analysis and regulation of a gene encoding a cuticle-degrading serine protease from the nematophagous fungus *Arthrobotrys oligospora*. Microbiology 142:1605–1616.

Åhman J, Olsson M, Johansson T, Punt PJ, van den Hondel CAMJJ, Tunlid A. 2002. Improving the pathogenicity of a nematode-trapping fungus by genetic engineering of subtilisin with nematotoxic activity. Appl Environ Microbiol. 68:3408–3415.

Alias SA, Kuthubutheen AJ, and Jones EBG. 1995. Frequency of occurrence of fungi on wood in Malaysian mangroves. Hydrobiologia 295:97016.

Anderson MG, Jarman TB, Rickards RW. 1995. Structures and absolute configurations of antibiotics of the oligosporon group from the nematode-trapping fungus *Arthrobotrys oligospora*. J Antibiot. 48:391–398.

Anderson MG, Rickards RW, Lacey E. 1999. Structures of flagranones A, B and C, cyclohexenoxide antibiotics from the nematode-trapping fungus *Duddingtonia flagrans*. J Antibiot. 52:1023–1028.

Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. 2001. Electrostatics of nanosystems: Application to microtubules and the ribosome. Proc Natl Acad Sci USA 98: 10037–10041.

Balogh J, Tunlid A, Rosen S. 2003. Deletion of a lectin gene does not affect the phenotype of the nematode-trapping fungus *Arthrobotrys oligospora*. Fungal Genet Biol. 39:128–135.

Barron GL. 1977. The Nematode Destroying Fungi. Ontario, Canada: Canadian Biological Publications Ltd. 140 p.

Barron GL. 1981. Predators and parasites of microscopic animals. In: Biology of Conidial Fungi. Vol. 2. New York: Academic Press.

Barron GL. 1992. Lignolytic and cellulolytic fungi as predators and parasites. In: Carroll, G.C., Wicklow, D.T. (Eds.), The Fungal Community: Its Organization and Role in the Ecosystem. New York: Marcel Dekker.

Barron GL. 2003. Predatory fungi, wood decay, and the carbon cycle. Biodiversity 4:3–9.

Bird J, Herd RP. 1995. In vitro assessment of two species of nematophagous fungi (*Arthrobotrys oligospora* and *Arthrobotrys flagrans*) to control the development of infective cyathostome larvae from naturally infected horses. Vet Parasitol 56:181–187.

Blaxter M, Robertson W. 1998. The cuticle. In: Perry RN, Wright DJ (Eds.) The physiology and biochemistry of free-living and plant-parasitic nematodes. Wallingford ( UK): CABI.

Bloemers GF, Hodda M, Lambshead PJD, Lawton JH, Wanless FR. 1997. The effects of forest disturbance on diversity of tropical soil nematodes. Oecologia 111:575–582.

Bonants P, Fitters P, Thijs H, den Belder E, Waalwijk C, Henfling J. 1995. A basic serine protease from *Paecilomyces lilacinus* with biological activity against *Meloidogyne hapla* eggs. Microbiology 141:775–784.

Bordallo JJ, Lopez-Llorca LV, Jansson H-B, Salinas J, Persmark L, Asensio L. 2002 Colonization of plant roots by egg-parasitic and nematode-trapping fungi. New Phytol. 154:491–499.

Borges-Walmsley MI, Walmsley AR. 2000. cAMP signalling in pathogenic fungi: control of dimorphic switching and pathogenicity. Trends Microbiol. 8:133–141.

Borrebaeck CAK, Mattiasson B, Nordbring-Hertz B. 1984. Isolation and partial characterization of a carbohydrate-binding protein from a nematode-trapping fungus. J Bacteriol. 159:53–56.

Kiziewicz B, Czeczuga B. 2003. Occurrence and morphology of some predatory fungi, amoebicidal, rotifericidal and nematodicidal, in the surface waters of Bialystok region. Wiad Parazytol. 49:281–291.

Brachmann CB, Sherman JM, Devine SE, Cameron EE, Pillus L, Boeke JD. 1995. The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. 9:2888–2902.

Cayrol JC, 1979. Mushroom crops protection: a new nematophagic fungus for pest control in mushroom crops. Inra Inst Nat Rech Agron. Assignee. Patent FR2402412-A1.

Chandrawathani P, Omar J, Waller PJ. 1998. The control of the free-living stages of *Strongyloides papillosus* by the nematophagous fungus, *Arthrobotrys oligospora*. Vet Parasitol. 76:321–325.

Chuankun X, Minghe M, Leming Z, Keqin Z. 2004. Soil volatile fungistasis and volatile fungistatic compounds. Soil Biol Biochem. 36:1997–2004.

Cooke RC. 1963. Succession of Nematophagous Fungi during the Decomposition of Organic Matter in the Soil. Nature 197:205–205.

Cox G, Kusch M, Edgar R. 1981. Cuticle of *Caenorhabditis elegans* its isolate and partial characterization. J Cell Biol. 90:7–17.

Dackman C, Nordbring-Hertz B. 1992. Conidial traps: a new survival structure of the nematode-trapping fungus *Arthrobotrys oligospora*. Mycol Res 96:194–198.

Dieterich C, Sommer RJ. 2009. How to become a parasite: lessons from the genomes of nematodes. Trends Genet. 25: 203–209.

Dijksterhuis J, Veenhuis M, Harder W, Nordbring-Hertz B. 1994. Nematophagous fungi: physiological aspects and structure-function relationships. Adv Microb Physiol. 36:111–143.

Drechsler C. 1933a. Morphological diversity among fungi that prey on nematodes. J Wash Acad Sci. 23:138–141.

Drechsler C. 1933b. Morphological features of some fungi that capture and kill nematodes. J Wash Acad Sci. 23:267–270.

Drechsler C. 1934. Organs of capture in some fungi preying on nematodes. Mycologia 26:134–144.

Duddington CL. 1954. Nematode-destroying Fungi in Agricultural Soils. Nature 173:500–501.

Farrell FC, Jaffee BA, Strong DR. 2006. The nematode-trapping fungus *Arthrobotrys oligospora* in soil of the Bodega marine reserve: distribution and dependence on nematode-parasitized moth larvae. Soil Biol Biochem. 38:1422–1429.

Fresenius G. 1852. Beitraege zur Mykologie. Heft 1–2:1–80.

Friman E, Olsson S, Nordbring-Hertz B. 1985. Heavy trap formation by *Arthrobotrys oligospora* in liquid culture. FEMS Microbiol Lett. 31:17–21.

Fuhrman JA. 2009. Microbial community structure and its functional implications. Nature 459:193–199.

Giovannoni SJ, Stingl U. 2005. Molecular diversity and ecology of microbial plankton. Nature 437:343–348.

Grønvold J, Wolstrup J, Nansen P, Henriksen SA. 1993a. Nematode-trapping fungi against parasitic cattle nematodes. Parasitol Today 9:137–140.

Grønvold J, Wolstrup J, Nansen P, Henriksen SA, Larsen M, Bresciani J. 1993b. Biological control of nematode parasites in cattle with nematode-trapping fungi: a survey of Danish studies. Vet Parasitol. 48:311–325.

Grewal PS, Sohi HS. 1988. A new and cheaper technique for rapid multiplication of *Arthrobotrys oligospora* and its potential as a bionematicide in mushroom culture. Curr Sci India 57:44–46.

Gunkel F, Gassen H. 1998. Proteinase K from *Tritirachium album* Limber. Characterisation of the chromosomal gene and expression of the cDNA in *Escherichia coli*. Eur J Biochem. 179:185–194.

Hashmi HA, Connan RM. 1989. Biological control of ruminant trichostrongylids by *Arthrobotrys oligospora*, a predacious fungus. Parasitol Today 5:28–30.

Hawksworth DL. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol Res. 105:1422–1432.

Hay FS, Niezen JH, Miller C, Bateson L, Robertson H. 1997a. Infestation of sheep dung by nematophagous fungi and implications for the control of free-living stages of gastro-intestinal nematodes. Vet Parasitol. 70:247–254.

Hay FS, Niezen JH, Ridley GS, Bateson L, Miller C, Robertson H. 1997b. The influence of pasture species and time of deposition of sheep dung on infestation by nematophagous fungi. Appl Soil Ecol. 6:181–186.

Heintz CE, Pramer D. 1972. Ultrastructure of nematode-trapping fungi. J Bacteriol. 110:1163.

Hoffmeister D, Kellerb NP. 2007. Natural products of filamentous fungi: enzymes, genes, and their regulation. Nat Prod Rep. 24:393–416.

Huang X, Zhao N, Zhang K. 2004. Extracellular enzymes serving as virulence factors in nematophagous fungi involved in infection of the host. Res Microbiol. 155:811–816.

Jaffee BA. 2004. Wood, nematodes, and the nematode-trapping fungus *Arthrobotrys oligospora*. Soil Biol Biochem. 36:1171–1178.

Jaffee BA, Strong DR. 2005. Strong bottom-up and weak top-down effects in soil: nematode-parasitized insects and nematode-trapping fungi. Soil Biol Biochem. 37:1011–1021.

Jairajpuri MS, Åhmad W. 1992. Dorylaimida: free-living, predacious and plant-parasitic nematodes. New Delhi: IBH.

Jansson H-B. 1982. Attraction of nematodes to endoparasitic nematophagous fungi. Trans Br Mycol Soc. 79:25–29.

Jansson H-B, Nordbring-Hertz B. 1981. Trap and conidiophore formation in *Arthrobotrys superba*. Trans Br Mycol Soc. 77:205–207.

Jansson H-B, Lopez-Llorca LV. 2001. Biology of nematophagous fungi. In: Misra JKH, Horn BW. (Eds.). Trichomycetes and Other Fungal Groups. Enfield (NH): Science Publishers.

Jansson H-B, Lopez-Llorca LV. 2004. Control of nematodes by fungi. In: Arora, D.K. (Ed.). Fungal Biotechnology in Agricultural, Food, and Environmental Applications. New York: Marcel Dekker.

Jensen C, Lysek G. 1991. Direct observation of trapping activities of nematode-destroying fungi in the soil using fluorescence microscopy. FEMS Microbiol Lett. 85:207–210.

José P-M, José AU, Johnson AD. 1999. Phenotypic switching in *Candida albicans* is controlled by a SIR2 gene. EMBO J. 18:2580–2592.

Joshi L, St Leger R, Bidochka M. 1995. Cloning of a cuticle-degrading protease from the entomopathogenic fungus, *Beauveria bassiana*. FEMS Microbiol Lett. 125:211–217.

Kerry BA, Jaffee BA. 1997. Fungi as biocontrol agents for plant parasitic nematodes. In: Wicklow DTS, (Ed.). The Mycota IV. Berlin: Springer.

Khan A, Williams K, HKM N. 2004. Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biol Control 31:346–352.

Kieu HV, Sidorova II, Gorlenko MV. 1971. Biologically active substance of predatory fungi. II. Antibiotic substances of predatory hyphomycetes of genera *Arthrobotrys*, *Dactylaria*, and *Dactylella*. Biologicheskie Nauki (Moscow) 14: 86–91.

Kobayashi DY, Crouch JA. 2009. Bacterial/Fungal interactions: from pathogens to mutualistic endosymbionts. Annu Rev Phytopathol. 47:63–82.

Koppenhöer AM, Jaffee BA, Muldoon AE, Strong DR, Kaya HK. 1996. Effect of Nematode-Trapping Fungi on an Entomopathogenic Nematode Originating from the Same Field Site in California. J Invertebr Pathol. 68:246–252.

Lee J-K, Kim D-G, Lee S-B. 2004. Nutritional Requirements and Mass Production of Nematode-trapping Fungus, *Arthrobotrys oligospora*. J Asia Pac Entomol. 7:325–329.

Li G, Zhang K, Xu J, Dong J, Liu Y. 2007a. Nematicidal Substances from Fungi. Recent Pat Biotechnol. 1:212–233.

Li J, Yang J, Huang X, Zhang K. 2006. Purification and characterization of an extracellular serine protease from *Clonostachys rosea* and its potential as a pathogenic factor. Process Biochem. 41:925–929.

Li L, Qu Q, Tian B, Zhang KQ. 2005a. Induction of Chlamydospores in *Trichoderma harzianum* and *Gliocladium roseum* by Antifungal Compounds Produced by *Bacillus subtilis* C2. J Phytopathol. 153:686–693.

Li L, Mo M, Qu Q, Luo H, Zhang K. 2007b. Compounds inhibitory to nematophagous fungi produced by *Bacillus* sp. strain H6 isolated from fungistatic soil. Eur J Plant Pathol. 117:329–340.

Li TF, Zhang KQ, Liu XZ. 2000. Taxonomy of Nematophagous Fungi. Beijing: Chinese Scientific and Technological Publications.

Li Y, Kevin D, Jeewon R, Cai L, Vijaykrishna D, Zhang K. 2005b. Phylogenetics and evolution of nematode-trapping fungi (Orbiliales) estimated from nuclear and protein coding genes. Mycologia 97:1034–1046.

Liang L, Meng Z, Ye F, Yang J, Liu S, Sun Y, Guo Y, Mi Q, Huang X, Zou C, Rao Z, Lou Z, Zhang K-Q. 2010. The crystal structures of two cuticle-degrading proteases from nematophagous fungi and their contribution to infection against nematodes. FASEB J. 24:1391–1400.

Liu XZ, Xiang MC, Che YS. 2009. The living strategy of nematophagous fungi. Mycoscience 50: 20–25.

Lopez-Llorca L, Olivares-Bernabeu C, Salinas J, Jansson H, Kolattukudy P. 2002. Prepenetration events in fungal parasitism of nematode eggs. Mycol Res. 106:499–506.

Luo H, Li X, GH L, Pan Y, KQ Z. 2006. Acanthocytes of *Stropharia rugosoannulata* function as a nematode-attacking device. Appl Environ Microbiol. 72:2982–2987.

Luo H, Mo M, Huang X, Li X, Zhang K. 2004. *Coprinus comatus*: a basidiomycete fungus forms novel spiny structures and infects nematodes. Mycologia 96:1218–1225.

Lysek G, Nordbringhertz B. 1981. An endogenous rhythm of trap formation in the nematophagous fungus *Arthrobotrys oligospora*. Planta 152:50–53.

McSorley R, Wang KH, Church G. 2008. Suppression of root-knot nematodes in natural and agricultural soils. Appl Soil Ecol. 39:291–298.

Mekhtieva NA, Kadzabova AA, Gasanova SC, 1980. Biological control of plant, e.g. oats, nematodes: using *Arthrobotrys oligospora* Fress-117 soil fungus strain as attractant. As Azerb Microbio, Assignee. Patent SU683701-A.

Mitreva M, Zarlenga DS, McCarter JP, Jasmer DP. 2007. Parasitic nematodes–From genomes to control. Vet Parasitol. 148:31–42.

Mo M-H, Chen W-M, Hao-RanYang, Zhang K-Q. 2008. Diversity and metal tolerance of nematode-trapping fungi in Pb-polluted soils. J Microbiol. 46:16–22.

Mo M-H, Chen W-M, Zhang K-Q. 2006. Heavy metal tolerance of nematode-trapping fungi in lead-polluted soils. Appl Soil Ecol. 31:11–19.

Moens M, Perry RN. 2009. Migratory plant endoparasitic nematodes: a group rich in contrasts and divergence. Annu Rev Phytopathol. 47:313–332.

Morton C, Hirsch P, Peberdy J, Kerry B. 2003. Cloning of and genetic variation in protease VCP1 from the nematophagous fungus *Pochonia chlamydosporia*. Mycol Res. 107: 38–46.

Nansen P, Gronvold J, Henriksen SA, Wolstrup J. 1988. Interactions between the predacious fungus *Arthrobotrys oligospora* and third-stage larvae of a series of animal-parasitic nematodes. Vet Parasitol. 26:329–337.

Nemecek JC, Wuthrich M, Klein BS. 2006. Global Control of Dimorphism and Virulence in Fungi. Science 312:583–588.

Nguyen VL, Bastow JL, Jaffee BA, Strong DR. 2007. Response of nematode-trapping fungi to organic substrates in a coastal grassland soil. Mycol Res. 111:856–862.

Nordbring-Hertz B. 1972. Scanning electron-microscopy of nematode-trapping organs in *Arthrobotrys oligospora*. Physiol Plant 26:279.

Nordbring-Hertz B. 1977a. Nematode-induced morphogenesis in predacious fungus *Arthrobotrys oligospora*. Nematologica 23:443–451.

Nordbring-Hertz B. 1977b. X-ray microanalysis of the nematode-trapping organs in *Arthrobotrys oligospora*. Trans Br Mycol Soc. 68:53–57.

Nordbring-Hertz B. 2004. Morphogenesis in the nematode-trapping fungus *Arthrobotrys oligospora* – an extensive plasticity of infection structures. Mycologist 18:125–133.

Nordbring-Hertz B, Mattiasson B. 1979. Action of a nematode-trapping fungus shows lectin-mediated host-microorganism interaction. Nature 281:477–479.

Nordbring-Hertz B, Jansson H, A T. 2000. Nematophagous fungi. In: Encyclopedia of life sciences. London: Macmillan.

Nordbring-Hertz B, Fehne-Persson K, De Vries OMH, Veenhuis M. 1989. Protoplast formation from adhesive network traps of *Arthrobotrys oligospora*. Mycol Res. 92:50–54.

Nordbring-Hertz B, Jansson H-B, Tunlid A. 2002. Nematophagous fungi. In: Encyclopedia of Life Sciences. London: Macmillan.

Nordbring-Hertz B, Jansson HB, Tunlid A. 2006. Encyclopedia of Life Sciences. Chichester: Wiley.

Nordbring-Hertz B, Neumeister H, Sjollema K, Veenhuis M. 1995. A conidial trap-forming mutant of *Arthrobotrys oligospora*. Mycol Res. 99:1395–1398.

Nordbring-Hertz B, Stalhammarcarlemalm M. 1978. Capture of Nematodes by *Arthrobotrys oligospora:* an electron microscope study Can J Bot. 56:1297–1307.

Olsson S, Persson Y. 1994. Transfer of phosphorus from *Rhizoctonia solani* to the mycoparasite *Arthrobotrys oligospora*. Mycol Res. 98:1065–1068.

Olthof THA, Estey RH. 1963. A Nematotoxin produced by the Nematophagous Fungus *Arthrobotrys oligospora* Fresenius. Nature 197:514–515.

Onofri S, Selbmann L, de Hoog GS, Grube M, Barreca D, Ruisi S, Zucconi L. 2007. Evolution and adaptation of fungi at boundaries of life. Adv Space Res .40:1657–1664.

Persmark L, Jansson H-B. 1997. Nematophagous fungi in the rhizosphere of agricultural crops. FEMS Microbiol Ecol. 22:303–312.

Persmark L, Nordbring-Hertz B. 1997. Conidial trap formation of nematode-trapping fungi in soil and soil extracts. FEMS Microbiol Ecol. 22:313–323.

Persmark L, Banck A, Andersson S, Jansson HB. 1992. Evaluation of methods for extraction of nematodes and endoparasitic fungi from soil. Nematalogica 38: 520–530.

Persmark L, Banck A, Jansson H-B. 1996. Population dynamics of nematophagous fungi and nematodes in an arable soil: vertical and seasonal fluctuations. Soil Biol Biochem. 28:1005–1014.

Persson Y, Nordbring-Hertz B, Chet I. 1990. Effect of polyoxin D on morphogenesis of the nematode-trapping fungus *Arthrobotrys oligospora*. Mycol Res. 94:196–200.

Peterson EA, Katznelson H. 1964. Occurrence of Nematode-trapping Fungi in the Rhizosphere. Nature 204:1111–1112.

Pramer D, Stoll NR. 1959. Nemin: Morphogenic substance causing trap formation by predaceous fungi. Science 129:966–967.

Premachandran D, Pramer D. 1984. Role of *N*-Acetylgalactosamine-Specific Protein in Trapping of Nematodes by *Arthrobotrys oligospora*. Appl Environ Microbiol. 47:1358–1359.

Román E, Arana DM, Nombela C, Alonso-Monge R, Pla J. 2007. MAP kinase pathways as regulators of fungal virulence. Trends Microbiol. 15:181–190.

Rosén S, Ek B, Rask L, Tunlid A. 1992. Purification and characterization of a surface lectin from the nematode-trapping fungus *Arthrobotrys oligospora*. J Gen Microbiol. 138:2663–2672.

Rosén S, Bergström J, Karlsson K-A, Tunlid A. 1996a. A multispecific saline-soluble lectin from the parasitic fungus *Arthrobotiys oligospora.* Similarities in the binding specificities compared with a lectin from the mushroom *Agaricus bisporus*. Eur J Biochem. 238:830–837.

Rosén S, Kata M, Persson Y, Lipniunas PH, Wikström M, van den Hondel CAMJJ, van den Brink JM, Rask L, Heden L-O, Tunlid A. 1996b. Molecular characterization of a saline-soluble lectin from a parasitic fungus Extensive sequence similarities between fungal lectins. Eur J Biochem. 238:822–829.

Rosén S, Sjollema K, Veenhuis M, Tunlid A. 1997. A cytoplasmic lectin produced by the fungus *Arthrobotrys oligospora* function as a storage protein during saprophytic and parasitic growth. Microbiology 143:2593–2604.

Rubner A. 1996. Revision of predacious hyphomycetes in the *Dactylella-Monacrosporium* complex. Stud Mycol. 39:1–134.

Satchuthananthavale V, Cooke RC. 1967. Carbohydrate Nutrition of some Nematode-trapping Fungi. Nature 214:321–322.

Saumell CA, Padilha T. 2000. Influence of weather and time of deposition on sheep faeces colonization by nematophagous fungi in the Mata region of Minas Gerais State, Brazil. Appl Soil Ecol. 14:63–70.

Saumell CA, Padilha T, Santos C, de P, Roque MVC. 1999. Nematophagous fungi in fresh feces of cattle in the Mata region of Minas Gerais state, Brazil. Vet Parasitol. 82:217–220.

Saxena G. 2008. Observations on the occurrence of nematophagous fungi in Scotland. Appl Soil Ecol. 39:352–357.

Sayers G, Sweeney T. 2005. Gastrointestinal nematode infection in sheep – a review of the alternatives to anthelmintics in parasite control. Anim Health Res Rev. 6:159–171.

Schenck S, TJ C, WD R, D P. 1980. Collagenase production by nematode-trapping fungi. Appl Environ Microbiol. 40:567–570.

Schultz R, Liebman M. 1997. Structure–function relationship in protein families. In: Devlin TM (Ed.). Textbook of biochemistry with clinical correlations. Fourth edn. New York: Wiley-Liss. 1–2 p.

Sharon N, Lis H. 1972. Cell-Agglutinating and Sugar-Specific Proteins. Science 177:949–959.

Sharon N, Lis-Sharon H. 1989. Lectins as cell recognition molecules. Science 246:227–234.

Shepherd AM. 1955. Formation of the Infection Bulb in *Arthrobotrys oligospora* Fresenius. Nature 175:475.

Sorokin N. 1876. Note sur Les vegetaux parasites des anguillulas. Ann Sci Nat Bot. 6:63.

St Leger R, Frank D, Roberts D, RC S. 1992. Molecular cloning and regulatory analysis of the cuticle-degrading protease structural gene from the entomopathogenic fungus *Metarhizium anisopliae*. Eur J Biochem. 204:991–1001.

Stadler M, Anke H, Sterner O. 1993a. Linoleic acid, the nematicidal principle of several nematophagous fungi and its production in trap-forming submerged cultures. Arch Microbiol. 160:401–405.

Stadler M, Sterner O, Anke H. 1993b. New biologically active compounds from the nematode-trapping fungus *Arthrobotrys oligospora* Fresen. Z Naturforsch C 48:843–850.

Su H, Hao Ye, Mo M, Zhang K. 2007. The ecology of nematode-trapping hyphomycetes in cattle dung from three plateau pastures. Vet Parasitol .144:293–298.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 24:1596–1599.

Tosi S, Annovazzi L, Tosi I, Iadrola P, G C. 2001. Collagenase production in an Antarctic strain of *Arthrobotrys tortor* Jarowaja. Mycopathologia 153:157–162.

Tunlid A, Jansson S. 1991. Proteases and their involvement in the infection and immobilization of nematodes by the nematophagous fungus *Arthrobotrys oligospora*. Appl Environ Microbiol. 57:2868–2872.

Tunlid A, Johansson T, Nordbring-Hertz B. 1991. Surface polymers of the nematode-trapping fungus *Arthrobotrys oligospora*. J Gen Microbiol. 137:1231–1240.

Tunlid A, Rosén S, Ek B, L R. 1994. Purification and characterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys oligospora*. Microbiology 140:1687–1695.

Tunlid A, Åhman J, Oliver RP. 1999. Transformation of the nematode-trapping fungus *Arthrobotrys oligospora*. FEMS Microbiol Lett. 173:111–116.

Veenhuis M, Nordbring-Hertz B, Harder W. 1984. Occurrence, characterization and development of two different types of microbodies in the nematophagous fungus *Arthrobotrys oligospora*. FEMS Microbiol Lett. 24:31–38.

Veenhuis M, Nordbring-Hertz B, Harder W. 1985a. Development and fate of electron dense microbodies in trap cells of the nematophagous fungus *Arthrobotrys oligospora*. Anton Leeuw Int J G 51:399–407.

Veenhuis M, Nordbring-Hertz B, Harder W. 1985b. An ultrastructural study of cell-cell interactions in capture organs of the nematophagous fungus *Arthrobotrys oligospora*. FEMS Microbiol Lett. 30:93–98.

Veenhuis M, van Wijk C, Wyss U, Nordbring-Hertz B, Harder W. 1989. Significance of electron dense microbodies in trap cells of the nematophagous fungus *Arthrobotrys oligospora*. Anton Leeuw Int J G 56:251–261.

Wachira P, Mibey R, Okoth S, Kimenju J, Kiarie J. 2009. Diversity of nematode destroying fungi in Taita Taveta, Kenya. Fungal Ecol. 2:60–65.

Wang H, Bun Ng T, Ooi VCE. 1998. Lectins from mushrooms. Mycol Res. 102:897–906.

Wang M, Yang J, Zhang K. 2006a. Characterization of an extracellular protease and its cDNA from the nematode-trapping fungus *Monacrosporium microscaphoides*. Can J Microbiol. 52:130–139.

Wang R, Yang J, Lin C, Zhang K. 2006b. Purification and characterization of an extracellular serine protease from the nematode-trapping fungus *Dactylella shizishanna*. Lett Appl Microbiol. 42:589–594.

Werthmann-Cliemas J, Lysek G. 1986. Formation of synnematous conidiophores in *Arthrobotrys oligospora*. Trans Br Mycol Soc. 87:656–658.

Wimmerova M, Mitchell E, Sanchez J-F, Gautier C, Imberty A. 2003. Crystal Structure of Fungal Lectin. J Biol Chem. 278:27059–27067.

Woronin M. 1870. *Sphaeria lemneae*, *Sordaria coprophila*, *Arthrobotrys oligospora*. Bettr. Morph. Physiol. Pilze. III. Senckenbergisch naturf Ges 7:325–360.

Yan X, Zheng F, Zheng J, Li R. 2007. Control of *Meloidogyne enterolobii* using HNQ11 Strain of *Arthrobotrys oligospora*. Re Dai Zuo Wu Xue Bao 28:98–101.

Yang J, Huang X, Tian B, Sun H, Duan J, Wu W, Zhang K. 2005. Characterization of an extracellular serine protease gene from the nematophagous fungus *Lecanicillium psalliotae*. Biotechnol Lett. 27:1329–1334.

Yang J, Tian B, Liang L, Zhang KQ. 2007a. Extracellular enzymes and the pathogenesis of nematophagous fungi. Appl Microbiol Biotechnol. 75:21–31.

Yang JK, Li J, Liang LM, Tian BY, Zhang Y, Cheng CM, Zhang KQ. 2007b. Cloning and characterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys conoides*. Arch Microbiol. 188: 167–174.

Yang JK, Liang LM, Zhang Y, Li J, Zhang L, Ye FP, Gan ZW, Zhang KQ. 2007c. Purification and cloning of a novel serine protease from the nematode-trapping fungus *Dactylellina varietas* and its potential roles in infection against nematodes. Appl Microbiol Biotechnol 75:557–565.

Yang JK, Ye FP, Mi QL, Tang SQ, Li J, Zhang KQ. 2008. Purification and Cloning of an Extracellular Serine Protease from the Nematode-Trapping Fungus *Monacrosporium cystosporium*. J Microbiol Biotechnol. 18: 852–858.

Yang Y, Yang E, An Z, Liu X. 2007d. Evolution of nematode-trapping cells of predatory fungi of the Orbiliaceae based on evidence from rRNA-encoding DNA and multiprotein sequences. Proc Natl Acad Sci USA 104:8379–8384.

Zhang K, Mo M. 2006. Flora fungorum sinicorum. Vol 33. Arthrobotrys et genera cetera cognate (Chinese). Beijing: Science Press.

Zhao M, MH M, Zhang K. 2004. Characterization of a neutral serine protease and its full-length cDNA from the nematode-trapping fungus *Arthrobotrys oligospora*. Mycologia 96:16–22.

Zopf W. 1888. Zur Kenntnis der Infektions-Krankheiten neiderer Thiere. Nova Acta Leop Acad Naturf Halle 52:7.