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Scientific paper

Taxonomic Diversity of Fungi Associated with Some PCN Populations from Serbia

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SUMMARY

Increased content of pesticides in food chain resulted in using microorganisms as agents of biological control. The potato cyst nematodes (PCN) - *Globodera pallida* and *G. rostochiensis* belong to the group of the most important parasites - the quarantine organisms. The external and internal area of a cyst harbor numerous fungal and bacterial species. The aim of this study was to identify antagonistic fungi associated with some PCN populations from Serbia. Fungal antagonists of potato cyst nematodes have not been previously investigated in our country. The diversity of PCN fungal antagonists is not reflected only at the species level but also at the level of higher taxonomic categories.

Keywords: Potato Cyst Nematodes (PCN); Fungi; Diversity; Biological control; Serbia

INTRODUCTION

Increased public concern over the significant presence of pesticides in food chain and its consequences resulted in the development of biological agents as alternative methods of control invasive organisms.

The potato cyst nematodes (PCN) - *Globodera pallida* and *G. rostochiensis* belong to the group of the most important parasites-the quarantine organisms and their control is complex due to their long persistance in soil.

The fungal antagonists of nematodes consist of a great variety of organisms which include the nematode-trapping or predacious fungi, endoparasitic fungi, parasites of nematode eggs and cysts and fungi which produce metabolites toxic to nematodes. The fungal spore has undergone some remarkable adaptation and specialization to capture or penetrate nematodes. Among the lower fungi, motile zoospores appear to have positive tropism toward nematodes. *Catenaria anguillulae* spores most often accumulate around the natural body orifices of the nematode cuticle (Mankau, 1980). *Nematophthora gynophila* causes total destruction of *Heterodera avenae* cysts in less than seven days (Kerry, 1980). *Verticillium chlamydosporium, Acremonium strictum* and *Fusarium oxysporum* are the main parasites in eggs of *H. schachtii* (Dackman and Nordbring-Hertz, 1985).

Flavipin, a low molecular weight compound, a metabolite of the fungus *Chaetomim globosum* is responsible for most of the nematode-antagonistic activity (Nitao et al., 2002). Combined application of *Paecilomyces lilacinus* and *Monacrosporium lysipagum* reduced 65% of *Heterodera avenae* cysts (Khan et al., 2006). There is a commercialised biopesticide based on *Paecilomyces lilacinus* (Kerry, 2000). In our country, fungal antagonists of potato cyst nematodes have not been investigated so far. The aim of the study was to identify taxonomic diversity of fungi associated with some PCN populations from Serbia.

MATERIAL AND METHODS

Mycobiota of *Globodera* cysts from Kladnica, Šanac, Gojna Gora and Milatovići populations which are morphologically and molecularly identified (Oro et al., 2010) was investigated. The cysts (3) and eggs of a single cyst were placed on potato-dextrose agar (PDA), containing antibiotics (bensylpenicillin K + bensylpenicillin-procaine: 200.000 i.u.+600.000 i.u., 200 mg/l), and incubated for two weeks at 25 a°C. The cysts and eggs were previously disinfected according to Heungens et al., 1996.

For identification purpose fungi were maintained on MEA (Malt Extract Agar) at room temperature. The isolates were identified by morpho-dimensional analysis using slide-cultures of the fungi. Only *Mycelia Sterilia* was identified by biomolecular analysis.

RESULTS AND DISCUSSION

The external and internal area of a cyst harbor numerous fungal and bacterial species competing for substrate. In the process of identification, the following fungal species were found: *Fusarium oxysporum* on cysts of the Kladnica population, *Oxyporus latemarginatus* on eggs of the same population, *Botrytis cinerea* and *Aspergillus fumigatus,* on eggs of the Šanac population, *Geotrichum candidum* on cysts of the Gojna Gora population, *Oxyporus latemarginatus* on eggs of the same population, *Geotrichum candidum* on cysts of the Milatovići population and *Aspergillus fumigatus* was isolated on eggs of the Milatovići population. The found species are harmless saprobes and they are relatively abundant members of the soil microbial community. Nearly all of them performed antagonistic activity toward potato cyst nematodes and have the potential to be used as biocontrol agents (results are not shown). The morphological view of isolated fungi is presented in photos.

Fusarium oxysporum Schlecht. Fr.

Fungi
Dikarya
Ascomycota
Pezizomycotina
Sordariomycetes
Hypocreales
Nectriaceae
Fusarium Teleomorph: *Gibberella*

Fusarium is a large genus of filamentous fungi widely distributed in soil and in association with plants (Ohara et al., 2004).

Figure 1. Mycelium of *Fusarium oxysporum* (PDA)

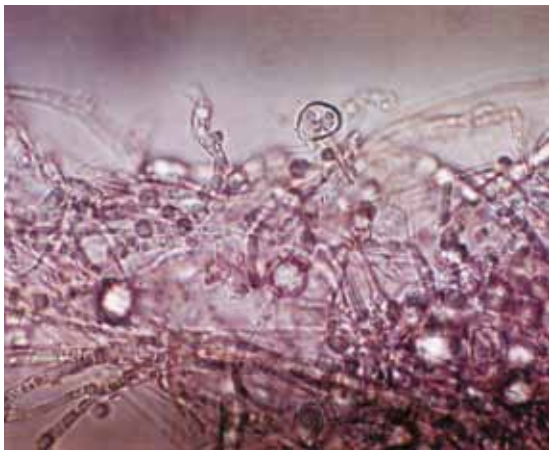

Figure 2. *Fusarium oxysporum* (LM 400X)

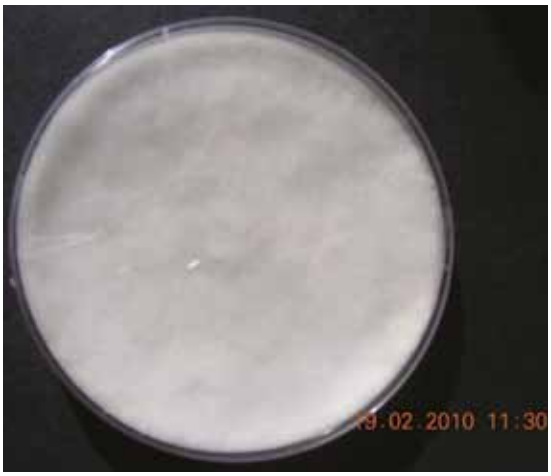

Figure 3. Mycelium of *Oxyporus latemarginatus* (PDA)

Figure 4. *Oxyporus latemarginatus* (Di 13X)

Many *F. oxysporum* isolates appear to be host specific, which has resulted in the subdivision of the species into *formae speciales* and races that reflect the apparent plant pathogenic specialization. *Fusarium oxysporum* and its various *formae speciales* have been characterized as causing the following symptoms: vascular wilt, yellows, corm rot, root rot, damping-off etc. (Kim et al., 2002). In solid media culture, such as potato dextrose agar (PDA), different special forms of *F. oxysporum* can have varying appearances. On PDA, growth is rapid and the white aerial mycelium may become tinged with purple or be submerged by the blue color of the sclerotia or by the cream to tan to orange sporodochia when they are abundant (Nelson et al., 1983; Leslie and Summerell, 2006). *Fusarium oxysporum* produces three types of asexual spores: microconidia, macroconidia, and chlamydospores (Ruiz-Roldan et al., 2010). Mycelium of *Fusarium oxysporum* on PDA and under a light microscope (LM) are shown in figures 1 and 2 respectively.

Oxyporus latemarginatus (Durieu and Mont.) Donk

Fungi
Dikarya
Basidiomycota
Agaricomycotina
Agaricomycetes
Polyporales
Polyporaceae
Oxyporus

Oxyporus latemarginatus is widely distributed in Europe; it induces stem and root rot of *Fagus* and *Quercus* species. The fungus colonizes and survives on dead wood or roots in the soil, forming white mycelial mats and dirty white to pale yellow fruiting bodies, annual, resupinate, rather soft when fresh, 2-5 (10) mm thick (Ryvard and Gilbertson, 1994). Mycelium of *Oxyporus latemarginatus* on PDA and under a dissecting microscope (Di) are shown in figures 3 and 4 respectively.

Botrytis cinerea (De Bary) Whetzel

Fungi
Dikarya
Ascomycota
Pezizomycotina
Leotiomycetes
Helotiales
Sclerotiniaceae
Botrytis Teleomorph: *Botryotinia*

Botrytis cinerea has a worldwide distribution but occurs mainly in humid temperate and subtropical regions; it is very common in the phylloplane of plants but does not attack healthy leaves; it can be facultative parasite on a wide range of plants causing blight or rot of leaves, flowers and fruits, the so-called gray mold (Domsch et al., 1980).

Figure 5. Mycelium of *Botrytis cinerea* (PDA)

Figure 6. *Botrytis cinerea* (LM 400X)

Figure 7. Mycelium of *Aspegillus fumigatus* (PDA)

Figure 8. *Aspegillus fumigatus* (LM 400X)

Botrytis cinerea causes infections characterized by rapid destruction (maceration, necrosis) of the tissues of its plant host as it colonies it (necrotrophy).

The fungus overwinter as mycelia or black resting structures called sclerotia that may be evident on dead plant tissue in late summer. Sclerotia germinate in the spring, or mycelium grows out of infected debris and conidia (infectious spores) develop (Williamson et al., 2007).

Mycelium of *Botrytis cinerea* on PDA and under a light microscope (LM) are shown in figures 5 and 6 respectively.

Aspergillus fumigatus Fresenius

Fungi
Dikarya
Ascomycota
Pezizomycotina
Eurotiomycetes
Eurotiales
Trichocomaceae
Aspergillus Teleomorph: *Emericella*

Aspergillus fumigatus, a saprotroph widespread in nature, is typically found in soil and decaying organic matter, where it plays an essential role in carbon and nitrogen cycling. Colonies of the fungus produce from conidiophores thousands of minute grey-green conidia (2-3 µm) that readily become airborne. For many years, *A. fumigatus* was thought to reproduce only asexually, as neither mating nor meiosis had ever been observed. O´Gorman et al., 2009 proved *A. fumigatus* possess a fully functional sexual reproductive cycle. The fungus is capable of growth at 37º C up to 50º C, with conidia surviving at 70º C. Mycelium of *Aspergillus fumigatus* on PDA and under a light microscope (LM) are shown in figures 7 and 8 respectively.

Geotrichum candidum Link

Fungi
Dikarya
Ascomycota
Saccharomycotina
Saccharomycetes
Saccharomycetales
Endomycetaceae
Geotrichum Teleomorph: *Dipodascus*

Geotrichum candidum is an extremely common fungus with a worldwide distribution, occurring mainly in soil, air, water, sewage, various plant substrates and very frequently on milk and milk products (Domsch et al., 1980). It can be plant pathogen that causes sour rot on peach, nectarine, tomato and carrot. Species of the genus *Geotrichum* produce chains of hyaline, smooth, one-celled, subglobose to cylindrical, slimy arthroconidia

(Gente et al., 2006). Mycelium of *Geotrichum candidum* on PDA and under a light microscope (LM) are shown in figures 9 and 10 respectively.

Figure 9. Mycelium of *Geotrichum candidum* (PDA)

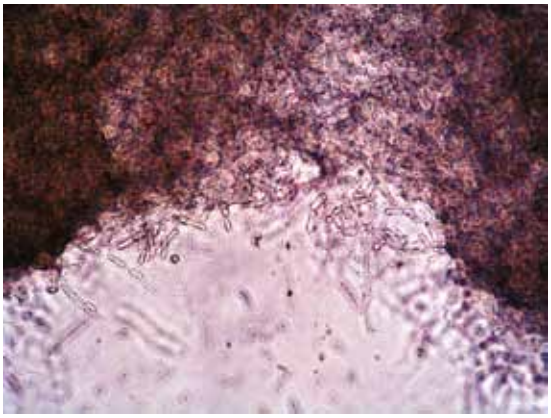

Figure 10. *Geotrichum candidum* (LM 400X)

The mode of antagonistic action of fungi on potato cyst nematodes is very complex and involves numerous toxins, metabolites, lytic enzimes and other antihelmintic compounds.

The main toxins produced by *Fusarium* species are fumonisins and trichothecenes (Embaby and Abdel-Galil, 2006). *Fusarium oxysporum* is a well known nematode antagonist used in many surveys (Morgan-Jones and Kabana, 1981; Bertrand et al., 2000; Athman et al., 2006; Daami-Remadi et al., 2009). Some strains are reported to be mycoparasites (Gupta et al., 1979), or parasites of weeds (Amsellem et al., 2001) and insects (Warma and Tandam, 1996). The fungus is a member of Nectriaceae family of Ascomycota phylum.

Oxyporus latemarginatus produces volatile, anti-fungal compound 5-pentyle-2-furaldehyde which is effective against *Alternaria alternata, Botrytis cinerea, Colletotrichum gloeosporoides, Magnaporthe grisea, Rhizoctonia solani, Fusarium oxysporum* f.sp. *lycopersici* (Lee et al., 2008). Pentyle-furaldehyde (PTF) was first reported as a nematicidal metabolite against *Aphelenchoides besseyi* from *Irpex lacteus* IFO5367 (Hayashi et al. 1981). Mayer et al. (1996) reported that PTF produced by an Ascomycete exhibits nematicidal activity against *Caenorhabditis elegans* and *Meloidogyne incognita*. *Oxyporus* belongs to Polyporaceae family and it is the only representative of Basidomycota.

Botrytis cinerea utilizes a large range of pathogenic factors (lytic enzymes, activated oxygen forms, toxins, plant hormones) to attack its host plants but it was not reported as nematode antagonist. The fungus is a member of Sclerotiniaceae family of Ascomycota.

When the fermentation broth of *Aspergillus fumigatus* was screened, a number of indolic alkaloids with antimitotic properties were discovered such as tryprostatins, with spirotryprostatin B being of special interest as an anticancer drug (Cui et al., 1996). *Aspergillus fumigatus* grown on certain building materials can produce genotoxic and cytotoxic mycotoxins, such as gliotoxin (Nieminen et al., 2002), it can also produce antibiotics such as sideramines fusigen, ferricrocin and fumigacin (Domsch et al., 1980). *Aspergillus fumigatus* had immobilized 51-75% juvenils of *Meloidogyne* spp. in three days (Mubyana-John and Wright, 2011). *Aspergillus* belongs to Trichocomaceae family of Ascomycota.

Insecticidal neoefrapeptins were isolated from *Geotrichum candidum* (Nagaraj et al., 2001). High egg parasitization (72%) of *Meloidogyne incognita* was observed by *Geotrichum candidum* (Goswami and Mittal, 2002). The fungus belongs to Endomycetaceae family or sometimes it is classified in Sacharomycetaceae, family of yeasts, within Ascomycota.

The process of cyst colonisation by fungi is enhanced by cystogenesis.

In shape, cysts are spherical sacs in which 500-600 eggs can be laid. There are specific ecological conditions within the cyst (temperature, relative humidity, pH, presence of different chemical compounds) which make it an ideal medium for microbial growth. Generally, eggs and larvae are not infected while cysts are intact. In process of hatching, the first larva appears leaving the vulva open and allowing fungal colonization. When detached from root tissue, mature cysts often lack a head leaving the cyst open on the other side, so that in this way antagonistic microorganisms can invade from both directions. In this study, different fungal species colonize external and internal side of a cyst. Although the majority of mentioned

fungal species are occasionally found in association with other nematode species, *Oxyporus latemarginatus* is for the first time found to be associated with PCNs. The diversity of PCN fungal antagonists is not reflected only at the species level but also at the level of higher taxonomic categories i.e. in existence of different phyla such as Ascomycota and Basidiomycota. Thus, the cyst can be considered as specific „microcosm" containing a spectrum of different fungi inside and outside of the cyst.

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Taksonomski diverzitet gljivica povezanih sa nekim populacijama CNK iz Srbije

REZIME

Povećan sadržaj pesticida u lancu ishrane je rezultirao korišćenjem mikroorganizama kao agenasa biološke kontrole. Cistolike nematode krompira (CNK) – *Globodera pallida* i *G. rostochiensis* spadaju u grupu najznačajnijih parazita – karantinskih organizama. Spoljašnjost i unutrašnjost ciste krije brojne vrste gljiva i bakterija. Cilj ovog rada je bio da se identifikuju gljivice u asocijaciji sa nekim populacijama CNK iz Srbije. U našoj zemlji fungalni antagonisti cistolikih nematoda krompira nisu bili istraživani do sada. Diverzitet fungalnih antagonista CNK se ne reflektuje samo na specijskom nivou, već i na nivou viših taksonomskih kategorija.

Ključne reči: Cistolike nematode krompira (CNK); gljivice; diverzitet; biološka kontrola; Srbija