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Occurrence and diversity of insect-associated fungi in natural soils in China

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ARTICLE INFO

Article history:
Received 28 January 2007
Received in revised form
23 November 2007
Accepted 5 December 2007

Keywords:
Insect pathogenic fungi
Opportunistic pathogenic fungi
Secondary colonizer
Community
Diversity
Soil
Mycoflora

ABSTRACT

In the present study, the occurrence and species diversity of insect-associated fungi in soil collected mainly from forest habitats in different regions of China were compared by using the '*Galleria* biat method'. Insect-associated fungi were defined to include known insect pathogenic fungi, opportunistic pathogens and secondary colonizers isolated from the *Galleria mellonella* bait insect exposed to the soil samples in question. Insect-associated fungi were detected in 55.5% of the 425 soil samples. A total of 377 fungi belonging to 46 species and 27 genera were isolated and identified. Among them, 6 species were known insect pathogenic fungi, 21 were opportunistic pathogens and 19 were secondary colonizers. Insect pathogenic fungi were most prevalent and *Paecilomyces farinosus*, *Beauveria bassiana* and *Metarhizium anisopliae* var. *anisopliae* (Hyphomycetes) were the most common species, comprising 19.6%, 14.1% and 10.6% of the total number of isolates, respectively. Opportunistic pathogens also had high occurrences in the soil with the percentage frequency added up to 36.9%. Among the opportunistic fungi, *Fusarium oxysporum*, *F. solani*, *Geomyces pannorum*, *Clonostachys rosea* f. *catenulata* and an unidentified *Fusarium* sp. resulted in the highest *G. mellonella* mortality in the preliminary pathogenicity test. Using principal component analysis, two components accounted for 76.5% of the total variance were extracted. Component 1 was positively correlated with species richness and species diversity and negatively correlated with the average altitude of the sampling region. Component 2 was negatively correlated with species evenness and positively correlated to the level of insect pathogenic fungi. The two-axis ordination of communities showed clear separation of the fungal community in South Central China, indicating higher occurrence of insect-associated fungi in the soil of subtropical humid region than the other regions.

1. Introduction

The soil habitat is considered as excellent habitat for insect pathogenic fungi and other microorganisms since it is protected from UV radiation and buffered against extreme biotic and abiotic influences (Keller and Zimmerman, 1989). Insect pathogenic fungi in the genera *Beauveria*, *Conidiobolus*, *Metarhizium* and *Paecilomyces* are all commonly found in the

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0929-1393/$ – see front matter
doi:10.1016/j.apsoil.2007.12.001

soil (Domsch et al., 1980). Many other fungal species have also been reported on diseased soil-inhabiting insects in various regions of the world and fungal epizootics in soil insect populations are also well documented (Samson et al., 1988; Keller and Zimmerman, 1989; Klingen and Haukeland, 2006).

To detect insect pathogenic fungi in soil, various selective media have been used (Veen and Ferron, 1966; Doberski and Tribe, 1980; Chase et al., 1986) which approximated the density of fungal propagules in soil. However, these selective media were developed only to several known species such as *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* var. *anisopliae* (Metschnikov) Sorokin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith. The '*Galleria* bait method' was first introduced by Zimmermann (1986) as a sensitive method to detect a broad spectrum of insect pathogenic fungi in soil samples. By comparing the two methods, Enkerli et al. (2004) found that *Beauveria brongniartii* (Sacc.) Petch isolates could be retrieved by '*Galleria* bait method' even when the fungus was at the level that could not be detected by using selective medium method. For those advantages, the '*Galleria* bait method' has been widely used in detection of insect pathogenic fungi in soil. The occurrence and distribution of insect pathogenic fungi in agricultural field soils have been extensively investigated in previous studies (Chandler et al., 1997; Bidochka et al., 1998; Ali-Shtayeh et al., 2002; Klingen et al., 2002; Keller et al., 2003; Meyling and Eilenberg, 2006). Some studies have also been conducted on the occurrence in adjacent hedgerows that are less affected by human cultivation activities (Klingen et al., 2002; Meyling and Eilenberg, 2006). These show a higher occurrence of insect pathogenic fungi in hedgerow and woodland soil. Higher density of insect pathogenic fungi was also founded in no-tilled soybean field soils than that under conventional tillage (Sosa-Gómez et al., 2001).

Other fungal species have also been found on insects in soil. During the study of the fungi from cadavers of the cave cricket *Troglophilus neglectus* Krauss (Rhaphidophoridae, Orthoptera), Gunde-Cimerman et al. (1998) found that *Mucor* spp., which have been considered as opportunistic pathogenic fungi, were isolated with the highest frequencies from the larval cadavers of *T. neglectus*. Species of the genera *Fusarium* and *Penicillium* have also been found in soil by using the '*Galleria* bait method' (Mietkiewski et al., 1991). Ali-Shtayeh et al. (2002) suggested that an essential weakly pathogenic fungus might at times become associated as the causal agent of epizootics in predisposed insects. Their preliminary pathogenicity test proved that isolates of the genera *Absidia*, *Aspergillus*, *Fusarium* and *Mucor* could kill the larvae of *Galleria mellonella* L. (Lepidoptera, Pyralidae). Actually, host insects in the soil are subject to challenge from many pathogen species. Avirulent pathogens have been reported to play significant role in insect-pathogen dynamics (Thomas et al., 2003). Therefore, we choose to include opportunistic fungi and secondary colonizers in our study of the naturally occurring fungi associated with insects in China. In using the '*Galleria* bait method', opportunistic pathogens can infect the weakened or wounded larvae during the baiting of soil samples and the secondary colonizers are those colonize the insect cadavers after the death of *G. mellonella*.

Gao et al. (1995) obtained *Beauveria* spp. and *Metarhizium* spp. from 78.7% of 47 soil samples collected in Northern China. This indicated high occurrence of insect pathogenic fungi in the soil in China. No detailed studies have been conducted on the occurrence of soil dwelling insect pathogenic fungi in China till this time. Further, the occurrence and diversity of insect pathogenic fungi in natural soils were not well documented. In this study, soil samples were collected in forestry and mountain habitats in different regions of China, the occurrence and distribution of insect-associated fungi were investigated by using the '*Galleria* bait method' (Zimmermann, 1986). To distinguish the relationship between the fungi and the insect, all fungi except the well-known insect pathogenic species were tested for their pathogenicity to *Galleria* larvae.

2. Materials and methods

2.1. Collection of soil samples

Soil samples were collected from different parts of China from the year 2003 to 2005. The samples were taken by cylindrical soil core borer (Φ = 20 mm) to a depth of 20 cm. Five columns of soil from an area about 5 m^2 were mixed as one sample. The samples were placed into plastic bags and stored at 4 °C. The samples were baited within 2 months.

A total of 425 soil samples originating from forests or mountains of 10 provinces in China were collected (Table 1). The sampling sites were grouped into six regions according to the climatic zone which they belonged to: Northern China included Dongling mountain and Wulingshan nature reserve; Western China included Linzhi, Mengda nature reserve and Beishan forestry center; Northeastern China included Liangshui national reserve and Changbai mountain; Middle-southern China included Tianmu mountain, Zhangjiajie national forest park and Jinggang mountain. Hainan Island included Wuzhi mountain, Jianfengling nature reserve and Diaoluo mountain; Himalayas, where soil samples were collected in Qomolangma Mountain above snow line.

2.2. Isolation and identification of fungi

Insect-associated fungi were isolated from soil samples by using the '*Galleria* bait method' (Zimmermann, 1986). The soil sample was passed through 2-mm pore sieve to remove plant tissues and molding gravels or blocks, and then mixed thoroughly and placed in a plastic bag. If the soil samples were too dry, they were moistened with sterile distilled water. Each of three autoclaved 50 mL centrifuge tubes was filled with soil of each sample and remained 1 cm of free air at the top of tube.

The wax moth, *Galleria mellonella* L. was reared continuously in constant darkness at 28 °C. The third or fourth instar larvae (approximately 30 days after hatching) were used as baits. Five larvae were placed on the soil surface in each tube and covered with lid, and then the samples were kept at the temperature of 20–25 °C for two weeks. During the first 4 days the tubes were upended twice a day to keep the larvae moving in the soil. The larvae were examined on 7th and 14th days after inoculation, respectively. Dead larvae were surface sterilized with 3% sodium hypochlorite for 3 min and then