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Research paper

Extracellular peptidases as possible markers of fungal ecology

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ABSTRACT

Fungi produce extracellular peptidases to break down proteins and polypeptides into smaller soluble molecules. These molecules are subsequently transported into the fungal cell, supplying osmotrophic nutrition. Although a variety of extracellular peptidases have been isolated from numerous fungal species, it remains largely unknown how the spectrum of secreted peptidases correlates with fungal ecology and taxonomy. In this study, we cultivated 17 fungal species on protein-enriched medium to compare the spectra of secreted peptidases produced by the fungi belonging to different ecological groups (pathogens, saprotrophs, symbionts) and taxonomic phyla (Ascomycota and Basidiomycota). Basidiomycetes predominantly produced metallopeptidases with neutral pH optima, while the species of ascomycetes secreted serine peptidases with alkaline pH optimum, mostly of the subtilisin-type. Fungal species with dominant activity of serine peptidases had, in general, much higher peptidase activity and were better adapted to growth on protein medium compared to the species with dominant metallopeptidase activity. Pathogenic fungi effectively cleaved the synthetic substrate specific for trypsin-like peptidases, while saprotrophic species were characterized by a relatively high aminopeptidase activity. Nevertheless, fungi have numerous genes encoding various types of peptidases, the spectra of fungal enzymes secreted on cultivation medium were to a major extent represented by the production of one class of peptidases that correlated with fungal taxonomic phylum. We suppose fungal strategies for cleaving polypeptides in experimental conditions are largely predetermined both by trophic status and phylogeny.

1. Introduction

Fungal secreted proteolytic enzymes and peptidase inhibitors are of great research interest for several reasons. These enzymes primarily support osmotrophic nutrition of the growing hyphae (Petrini et al., 1992; St. Leger et al., 1997), and secondly, peptidases play key roles in penetration of pathogenic fungi through the tissues of the host organism (St. Leger, 1995; Larcher et al., 1996; Karkowska-Kuleta et al., 2009; Yike, 2011). In addition, fungal peptidases and their inhibitors have increasing potential for practical application in medicine, agriculture, and technology (Rao et al., 1998; Raghukumar, 2008; Dunaevsky et al., 2014; De Souza et al., 2015).

The spectrum of secreted metabolites largely depends on the substrate that serves a fungus as a nutrition source. Thus, depending on the substrate, groups of saprotrophic and pathogenic fungi are described. The former feed on dead organic matter, including organic content of soil, compost, dead tissues of plants and animals, wood, harvested agricultural products, etc. According to specialization for specific substrate, saprotrophic fungi are further divided into different ecological groups (e.g. wood saprotroph, soil saprotroph, etc.). As opposed to saprotrophic species, pathogenic fungi spend at least part of their life cycle growing on the surface or inside other living organisms, and they feed on the living cells of the host. In addition to parasitic or saprotrophic lifestyles, fungi may have mutualistic relations with plants or animals, including examples of mycorrhiza and fungus gardens. For most fungal species, such division into symbionts, saprotrophs, and pathogens is, however, arbitrary due to ambiguity of fungal trophic lifestyles: most pathogenic species have saprotrophic feeding stages throughout their lifecycles, and saprotrophs are often known to colonize weakened living hosts.

Among fungal secreted hydrolases, including peptidases, the enzymes of pathogenic species are studied better than those of

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saprotrophs, even though by producing hydrolytic enzymes saprotrophic fungi decompose and mineralize organic matter (Colpaert and Van Tichelen, 1996; Wu et al., 2005), which is of high importance for the global nutrient turnover. Studying peptidases of pathogens relates to their practical importance, as well as key roles of secreted peptidases in infecting plants and animals, including humans (St. Leger, 1995; Larcher et al., 1996; Karkowska-Kuleta et al., 2009; Yike, 2011). The need to penetrate host tissues and to deactivate the defense barriers of the host (including enzymes and inhibitors) likely determines a broader spectrum of peptidases generally secreted by pathogenic fungi. In addition, saprotrophic species are generally characterized by relatively low peptidase activity, which is more difficult to study. Comparative analysis of fungal genomes revealed that saprotrophic species had fewer genes encoding secreted peptidases compared to the pathogens (Soanes et al., 2008).

Despite the limitations related to low secreted activity, there are numerous studies addressing specific secreted peptidases of saprotrophic and mycorrhizal fungi. Thus, proteolytic enzymes have been studied for the edible saprotrophic mushroom *Agaricusbisporus*, from which a serine peptidase with narrow substrate specificity was isolated (Burton et al., 1993). Secreted peptidases belonging to different classes and having various properties have been characterized for ectomycorrhizal basidiomycetes *Amanita muscaria, Lacaria laccata*, and *Suillus bovinus* (Chalot and Brun 1998; Mucha et al., 2007), the xylotrophic fungus *Hypsizygusmarmoreus* (Zhang et al., 2010), and the wood saprotroph *Pleurotus citrinopileatus* (Cui et al., 2007). Aspartyl peptidases were isolated from the ericoid mycorrhizal species *Hymenoscyphusericae* (Leake and Read, 1990).

Besides the culturing methods, with the development of molecular techniques more than a hundred of fungal genomes have been sequenced, revealing a vast diversity of enzyme families (Mohanta and Bae, 2015) and showing great potential of fungi for producing numerous peptidases. On the other hand, synthesis and secretion of peptidases is an energy-consuming process (Schimel, 2003), implying fitness costs involved in production of extracompounds. Therefore, fungi belonging to different ecological groups are expected to produce different enzymes reflecting the adaptation of the fungus to growing on a particular substrate. Thus, various mechanisms of enzymatic wood degradation leading to a yellowish or a brownish color of a wood residue are known for white- and brown-rot basidiomycetes. The white-rot basidiomycetes degrade both lignin and wood polysaccharides (celluloses and hemicelluloses), while brown-rot fungi rapidly break down wood celluloses and hemicelluloses but can only modify lignin by demethoxylation (Rytioja et al., 2014). In soil fungal communities, Talbot et al. (2013) reported a strong effect of fungal ecological function on the overall enzymatic soil profile. Richness in ectomycorrhizal species explained variation in the activity of enzymes targeting recalcitrant N sources, i.e. peptidases and peroxidases, while community composition of saprotrophic fungi correlated with activities of carbohydrate- and organic P-targeting enzymes (Talbot et al., 2013). Other studies referred to fungal phylogeny as a key factor controlling secreted enzyme profile. For example, Kuuskeri et al. (2015) reported various profiles of secreted manganese peroxidases and oxidoreductases observed across ten species of the genus *Phlebia*. Correlations found in that study predicted the spectrum of secreted enzymes for new *Phlebia* species, simply based on the position of the fungus on the phylogenetic tree. An opposite trend was reported by Rineau and Courty (2011), who found no correlation between enzymatic profiles and taxonomic lineages (genera, family of phylogenetically related groups) in ectomycorrhizal fungi, even though a strong correlation between fungal taxonomic diversity and enzyme activities in bulk soils were observed. On the other hand, comparing results of field- and culture-based measurements of enzyme activity may not be fully comparable because of the surrounding community affects the spectra of fungal metabolites.

In this study, we cultured 17 fungal species on a sterile protein-enriched medium to test whether the spectrum of fungal secreted peptidases correlates with any of the fungal traits (trophic status, taxonomic group). If the fungal secreted profile is primarily determined by the substratewe expected to observe largely similar (optimal for the given conditions) peptidase profiles for all the studied species. If spectra of secreted peptidases were predetermined by fungal ecology and taxonomic position, we expected to observe largely different enzyme profiles secreted by specific groups of fungi into the culturing medium.

2. Materials and methods

2.1. Fungal strains

Seventeen fungal species including 6 basidiomycetes and 11 ascomycetes were used in the present study (see Table 1 for the list of the species and their ecology and taxonomy). The non-symbiotic strains of several asco- and basidiomycetous fungi, including pathogens of plants and insects and saprotrophic fungi, were obtained from the All-Russian Collection of Microorganisms and the collection of the Department of Mycology and Algology, Moscow State University, Russia. The taxonomic identities of all

Table 1
Model fungi, their taxonomic affinities and trophic status.

Fungal species (strains)	Taxonomic affinity	Ecological function
Agaricus bisporus P77	Basidiomycota, Agaricales	saprotroph
Beauveria bassiana VKM 2274	Ascomycota, Hypocreales	insect pathogen
Botrytis cinerea VKM 3850	Ascomycota, Helotiales	plant pathogen
Coprinus comatus ZBC 571	Basidiomycota, Agaricales	saprotroph
Cordyceps militaris VKM-02-05	Ascomycota, Hypocreales	insect pathogen
Fusarium graminearum VKM 2306	Ascomycota, Hypocreales	plant pathogen
Humicol agrisea VKM 3846	Ascomycota, Sordariales	saprotroph
Leucoagaricus gongylophorus	Basidiomycota, Agaricales	saprotroph/ant symbiont
Marasmius oreades ZBC167	Basidiomycota, Agaricales	saprotroph
Metarhizium anisopliae VKM 1490	Ascomycota, Hypocreales	insect pathogen
Paecilomyces carneus VKM4010	Ascomycota, Eurotiales	sapotroph/soil
Penicillium nalgiovense VKM 229	Ascomycota, Eurotiales	saprotroph
Penicillium vulpinum VKM 2359	Ascomycota, Eurotiales	saprotroph
Pholiota aurivella ZBC211	Basidiomycota, Agaricales	saprotroph/wood
Pleurotus ostreatus 38d	Basidiomycota, Agaricales	saprotroph/wood
Trichoderma saturnisporum VKM 3245	Ascomycota, Hypocreales	saprotroph, mycopathogen
Trichurus spiralis VKM 3004	Ascomycota,Microascales	saprotroph