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# Proteomics of survival structures of fungal pathogens

Review

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**Fungal pathogens are causal agents of numerous human, animal, and plant diseases. They employ various infection modes to overcome host defense systems. Infection mechanisms of different fungi have been subjected to many comprehensive studies. These investigations have been facilitated by the development of various '-omics' techniques, and proteomics has one of the leading roles in this regard. Fungal conidia and sclerotia could be considered the most important structures for pathogenesis as their germination is one of the first steps towards a host infection. They represent interesting objects for proteomic studies because of the presence of unique proteins with unexplored biotechnological potential required for pathogen viability, development and the subsequent host infection. Proteomic peculiarities of survival structures of different fungi, including those of biotechnological significance (e.g., *Asperillus fumigatus*, *A. nidulans*, *Metarhizium anisopliae*), in a dormant state, as well as changes in the protein production during early stages of fungal development are the subjects of the present review. We focused on biological aspects of proteomic studies of fungal survival structures rather than on an evaluation of proteomic approaches. For that reason, proteins that have been identified in this context are discussed from the point of view of their involvement in different biological processes and possible functions assigned to them. This is the first review paper summarizing recent advances in proteomics of fungal survival structures.**

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## Introduction

Fungi are widespread eukaryotic organisms, classified as a kingdom, and include yeasts, molds and mushrooms as the most known representatives (Fig. 1). An overall estimation of fungal organisms varies between 1.5 million [1] and 5.1 million [2] species, but only around 100,000 of them have been described [3]. Among all fungal diversity, pathogenic fungi have recently attracted more attention of different research groups, what is documented by a constantly growing number of the corresponding publications in the PubMed database (since 2007 more than 2000 studies have been published annually).

There is a number of human, animal, plant and insect diseases caused by pathogenic fungi. Pathogens have a variety of attack modes including the secretion of cell wall degrading enzymes [4], effectors [5], toxins [6,7], etc. Developing effective strategies for antifungal protection requires the knowledge of the life cycle of a particular fungus and its virulence factors involved in the pathogenesis.

At the molecular level proteomics is currently one of the essential approaches for the characterization of structure and function of all living organisms, including fungi. Fungal proteome investigations are facilitated by growing results of genome sequencing projects, improvements in the performance of mass spectrometers, and the availability of user friendly software to analyze thousands of spectra in a matter of minutes. A lot of fungal species including for example filamentous fungi from the genera *Aspergillus* [8,9], *Botrytis* [10,11] and *Trichoderma* [12,13] or the basidiomycete *Trametes hirsuta* [14], have been subjected to proteomic studies. Most of these studies have been focused on the analysis of the respective fungal secretome and/or mycelium proteome. However, during the last 10 years, the attention of researchers was also attracted by conidial proteomes (Table 1). Fungal conidia are considered the main source of disease dissemination [15]. Their adhesion and germination on host surfaces represent crucial steps preceding the invasion and colonization, for which both sensing and recognition of the host surface characteristics, including hydrophobicity and sugar sources, seem essential [16,17]. Moreover, physical and chemical factors influence the virulence and stress tolerance of the conidia [18,19]. Therefore, studying conidial proteomes is an interesting and challenging task.

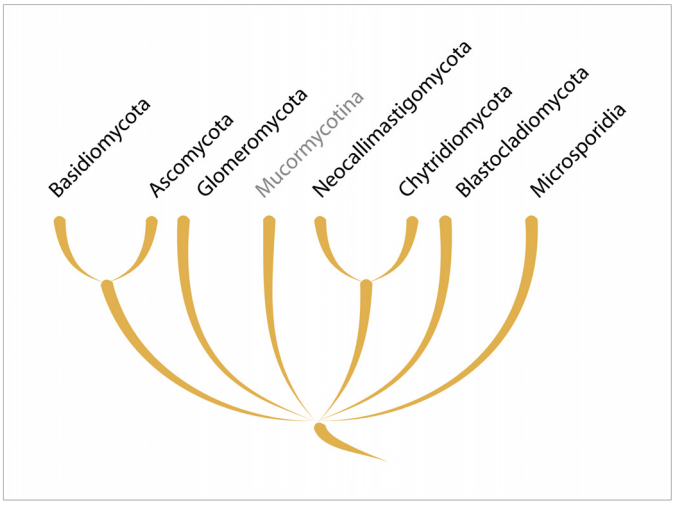


**FIGURE 1**

The phylogeny of fungi. The name in grey indicates fungi formerly known as 'Zygomycota' (created by Sabrina Setaro, distributed under a Creative Commons License CC BY 3.0).

A lot of fungi, including different pathogens, are widely implemented in the biotechnology industry, due to production of various enzymes, antibacterial and antifungal agents, and other metabolites [20]. Investigation of their conidial proteomes may allow identification of new enzymes of industrial interest, for example, different glycosyl hydrolases and proteases. Determination of key proteins of pathogen early development (e.g., germination) could be applied for creating of pathogen resistant plants, elaboration of new therapeutic agents, etc. For example, eco-friendly insecticides based on the proteins from enthomopathogenic fungi might be used in agriculture instead of chemical analogues [21].

In this review, we summarize results pertinent to the proteomes of the survival structures of pathogenic fungi to show the importance of these investigations for better understanding of the processes of fungal development and pathogenesis. We focus on proteomic analyses of dormant conidia, sclerotia, and changes of the proteomes during conidia germination. In addition, comparisons of mycelial versus conidial proteomes, and conidial surface-associated proteins are discussed. Further, specific characteristics of the protein content in conidia and sclerotia are described aiming to highlight important biological processes and their changes during fungal development. New virulence factors and possible protein markers are reviewed as well.

## Overview of experimental approaches

Different proteomic approaches have been applied for investigation of fungal survival structures. Nevertheless, the major steps of proteomic workflow remained the same: (i) fungi cultivation and harvesting of survival structures; (ii) disruption of survival structures; (iii) protein extraction; (iv) protein separation; (v) MS analysis and data interpretation.

All fungi were grown under appropriate conditions for production of survival structures. Solid state cultivation on different agar media has been implemented to this purpose. Among the pathogens included in the present review, only *Uromyces appendiculatus* [22] and *Blumeria graminis* [23,24] were grown on host plants, and *Nomuraea rileyi* [25] was grown on the surface of dead silkworm. Grounding in the liquid nitrogen and bead beating were techniques of choice for cell disruption, while trichloroacetic acid/acetone precipitation was the most popular extraction protocol. It should be mentioned that overviewed studies have been focused mainly on the investigation of a whole proteome rather than subcellular proteomes.