



Perspectives of Secretome Proteomics in Filamentous Fungi

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Filamentous fungi are eukaryotic protein factories having greater relevance in the modern day food and pharmaceutical industries. They are known for their astounding potential towards biodegradation and mineralization of xenobiotics and lignocellulosics. This unique Kingdom of organisms play an inevitable role in environmental cleanup and make up, adapting themselves by way of regulating their bound and secretory proteins. They make changes in the proteome composition and provide exciting opportunities in food, feed, biofuel, pharmaceutical and agro-industries. Overview of proteomic strategies in filamentous fungi with respect to extracellular proteomes, enzymes or secretomes will have greater relevance to understand the bio-based processes, such as biomass deconstruction and mycoremediation, wherein fungi are primarily involved. Scientists of "Proteogenomics Era" try to use customized protein sequence databases to identify novel peptides in filamentous fungi. Proteomics techniques can help to elucidate complex biological processes and are considered as powerful tools for the sensitive detection and rapid identification of filamentous fungi through protein expression understanding the interaction between fungal pathogen and host plant is more challenging. The expression of protein profiles in such cases is much complicated due to the presence of proteomes of two different species simultaneously, either at the infection court or at a remote place inside the partnering organisms. When the plant and its associated fungal biome is subjected to genomic analysis, the power of proteomics remains to be the chosen tool to unravel the molecular pathways of pathogenicity and resistance. Sometimes, in a compatible partnership like in mycorrhiza, the proteomics tools and techniques help to understand the symbiotic relationship. Experimental designs have also progressed extensively towards organelle proteomics and the knowledge on localization of proteins is of overriding importance to understand functions of various proteins at sub-cellular level. Organelle proteomics has gained insights in to compartmentalization and localization that exists in a fungal cell with respect to protein secretomes (e.g. production and process control of antibiotics, organic acids, enzymes etc.). Bioinformatics tools can help in the quantitative and qualitative estimation of such proteins to look beyond harnessing the full potential of filamentous fungi.

Key words: Fungi, Intracellular Proteins, Extracellular secretomes, Proteomics, Perspectives

"Animals eat their food and then digest it; fungi digest their food and then eat it" said Bennett (2006), which illustrates the vast number of extracellular enzymes necessarily produced by fungi to assimilate their food. The Fungal kingdom includes single celled yeasts to highly evolved moulds and mushrooms. It is the largest group of organisms with more than 500,000 described species and somewhere, one million might exist (Hawksworth, 1991). Over 8,000 species of fungi are known to cause plant diseases, sometimes creating great havocs (caused by *Phytophthora infestans*), stinking bunt of wheat caused by *Tilletia* spp, downy mildew of grapes caused by *Plasmopara viticola*). Irish famine in Europe during 1870s and Bengal famine in India during 1945 resulted in human sufferings and large scale human migrations (Ellis *et al.*, 2008). Clear examples of global economic implications brought out by fungi

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over mankind and also their interactions with plants and animals give important clues to be faced up for exploration of proteomics of filamentous fungi. The antiquity of this group dates back 550 million years, during which three major phyla viz., Zygomycota, Ascomycota and Basidiomycota are known to have diverged from Chytridiomycota. Infact, this evolution is said to have happened well before the actual land invasion by plants. In spite of their long years of existence, enigmatically, only a few fungi have been subjected to complete genome sequence. During 1996, the first completed genome sequence of yeast fungus, *Saccharomyces cerevisiae* was released. Early, in 2005 the edible fungus *Pleurotus sapidus* was subjected to secretome analysis and the data supported the interests of a methodological review of i) intracellular, ii) extracellular iii) membrane and organelle proteomes of filamentous fungi. Fungi can be easily cultured and mass multiplied through solid or liquid state fermentation. Their capacity to yield definite information on protein identity is also

well established. Moreover, localization and post-translational modification of proteins and accurate *in silico* gene modelling have been proved successful in several cases. The Fungal Genomics group at the Broad Institute has sequenced and analyzed a wide diversity of fungal organisms that are important to medicine, agriculture and industry. As of April, 2017 over 100 fungi including human and plant pathogens have been sequenced and the data released in public domain. These sequencing data support the incredible increment of proteomics applied manuscripts related to filamentous fungi. (<http://1000.fungalgenomes.org/home/>). The 'Central Dogma of Life' reveals that the DNA consist a specific sequence as gene, that can transcript into mRNA by the action of RNA polymerase and then translated into a protein by the action of ribosomes. Thus, a protein is the end product of a gene and plays a functional role in the organisation and characterisation of any organism. The study of that particular genome is called as proteomics. Proteomics has become an integral component of all large-scale "omic" and systems approaches to understand the complexity of fungal biochemistry (Doyle, 2011).

Fungi and white biotechnology

White biotechnology or industrial biotechnology refers to the use of living cells and / or their secretomes to create industrial products that are more easily degradable, requiring less energy and creating less waste during production. With the soaring energy prices, fear towards energy security and concern towards fast depletion of environment, white biotechnology is more important than ever. This is the next industrial co-evolutionary step towards a sustainable and environmentally benign chemical manufacturing process, empowering with the additional knowledge gained through genomics, proteomics, molecular genetics, metabolic engineering, catalysis, enzymology, solid and liquid state fermentation technology. White biotechnology works by making living cells into micro-factories, more meaningfully mushroom fungi are regarded as protein factories addressing human, animal and plant health. The Netherlands based global leader in white biotechnology, DSM with a fermentation capacity of over 30 million cubic metres per year states that bio-succinic acid can be applied for the production of agricultural films that can be 'shredded' and ploughed back into the soil after usage. These films are biodegradable within two months (https://www.dsm.com/content/dam/dsm/cworld/en_US/documents/white-biotech.pdf). Fungi are widely used in the process of enzyme extraction and lingo-cellulosics management. Filamentous fungi also have an unbeatable ability to secrete incalculable proteins, secondary metabolites and organic acids in culture medium. Sangeetha and Krishnamoorthy (2017) have extracted and purified an antifungal metabolite, cordycepin from cell free culture filtrate and mycelial mat extract of *Ophiocordyceps sinensis* and *O. neovolkiana*. In addition to the

control of *Fusarium* spp, the metabolite fraction also showed antinemic properties. The extracellular proteome analysis of submerged cultures of *Aspergillus oryzae* at different stages of growth contain impressive number of enzymes related to cell wall degradation viz., glucoamylase A, xylanase G2, glucosidase A, cellulase B, amylase, glucosidase and xylanase F3 (Machida *et al.*, 2005 and Oda *et al.*, 2006). Similarly, some of the secreted proteins of *Penicillium chrysogenum* (isoamyl alcohol oxidase, sulfhydryl oxidase, dihydroxyacid dehydratase, polygalacturonases, pectate lyases, ferulic acid esterases) are especially relevant in food, beverage and nutraceutical industries (Jami *et al.*, 2010b).

Quorn™ a kind of mycoprotein is made by the addition of oxygen, nitrogen, glucose and minerals to *Fusarium venenatum* in a fermenter to form a continuous supply of mycoprotein, harvested and dried before egg albumen is added for binding. Since 1967 almost 3,000 fungi have been tested before choosing *Fusarium graminearum* (culture A3/5) reclassified as *Fusarium venenatum* (PTA 2684) and the first Quorn™ products came to market in the 1980s. The first ever mycoprotein retail product was a vegetable pie made available in 1985. Now, there are over 90 products available in the Quorn range, including meals, grills, sausages, burgers and deli slices as well as cooking ingredients like mince, strips and pieces. The production of one tonne of Quorn™ mince and pieces requires just 15 % of the land required to produce one tonne of beef. Additional benefit could be lower levels of greenhouse gas emissions. Quorn™ products are relatively low in free sugars is also a positive effect (<http://www.mycoprotein.org/index.php/faq/index.html>). Due to their quality protein, alkaline ash, high potassium : sodium ratio and increased fibre, mushrooms are known to contain immunomodulatory biological response modifiers (BRM) addressing several health related issues including anticancer effect, anti HIV, reducing hypertension, hyperacidity and constipation. Fungal proteins are also known as biomarkers of pathogenicity in human and plants, also for the control of insect pests (Carlos Barreiro *et al.*, 2012).

Secretome proteomics of phytopathogenic fungi

Secretomes are complex molecules secreted by living organisms (Makridakis and Vlahou, 2010). Most of these molecules are freely released proteins, sometimes they may also be associated with outer cell wall. Many fungi secrete a huge number of proteins to facilitate their saprotrophic lifestyles. Hence, study of such molecules may help to establish the life cycles of such forms of life. Tjalsma *et al.* (2000) proposed even the cellular machinery involved in the protein secretion as part of secretome. Greenbaum *et al.*, (2001) indicated secretomes as functions of gene products. The recognition plant-pathogen interaction may be general or host specific. For example the *Botrytis cinerea* has wide host range and it can infect more than 200 hosts. It is also known to be a post harvest pathogen, more

specifically in mango and banana, infecting the fruits during storage and handling inflicting serious economic losses (Fernández-Acero *et al.*, 2010). The infection strategy by *B. cinerea* is mainly facilitated by a group of pathogenicity or virulence factors, which include peculiar pathogenicity related (PR) proteins (Shah *et al.*, 2009 and Espino *et al.*, 2010). These proteins were identified as pectin methyl esterases, xylanases and proteases which had a definite role in the degradation of plant defensive barriers. This finding is important in the sense of discovering newer pathogenicity factors to unravel the mechanism of pathogen infection. Two dimensional (2D) electrophoresis application combined with *de novo* sequencing and BLAST similarity search by MALDI-TOF Analyzer led to the protein identification of virulence factors (Cobos *et al.*, 2010). *Trichoderma* spp are known to be the best cellulose degraders and very often referred as mycoparasites on several plant pathogenic fungi. Suárez *et al.* (2005) using proteomics approach identified more than 250 extracellular proteins by serving chitin as carbon source. Monteiro *et al.* (2010) employed 2D gel electrophoresis and mass spectrometry to identify the mycelium degrading proteins secreted by the over growing colony of *Trichoderma harzianum* on plant pathogenic fungi like *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* sp. Enigmatically, the hyphae of *T. harzianum* was not found to coil the hyphae of *Fusarium* sp. revealing the fact that the secretomes related to mycoparasitism and cell wall degradation are entirely different. Metabolic enzymes like μ -mannosidase, exo-b-1,3-glucanase, heat-shock and BiP proteins have been found to be up-regulated in the virulent races compared to avirulent races of several fungal pathogens; such differences, only could reflect an adaptation behaviour of saprophytic existence in avirulent isolates, which may have significant applications in biological control of plant pathogens displacing virulent ones (Cao *et al.*, 2009). Gao *et al.* (2011) published the genome sequence of *Metarhizium anisopliae* and *Metarhizium acridum*, both entomopathogenic fungi used as alternatives to insecticides. Murad *et al.* (2006) reported about the secretion of proteases, reductases and acetyltransferases by *M. anisopliae* in the presence of the exoskeleton of *Callosobruchus maculatus*. Barros *et al.* (2010) observed clear differences in the expression of identical proteins and isoforms from conidia and mycelia of *M. anisopliae*. This kind of secretome analysis of entomopathogenic fungi is relatively poorly explored, which could offer prospering proteomics challenges in pest control programmes.

Moolighting proteins in fungi

Eukaryotic pathogens possess a number of virulence factors, but a relatively recently recognized and particularly interesting group of factors capable of enhancing virulence is the set of so-called 'moonlighting proteins'. These proteins include large collection of housekeeping enzymes lacking

special targeting motifs that would determine their extracellular localization, but that are often present at the cell surface of pathogen. Several such enzymes with key metabolic functions in glycolysis, the pentose phosphate cycle or other fundamental intracellular processes perform entirely new, non-catalytic roles often associated with adhesion to host ligands (Karkowska-Kuleta and Kozik, 2014). Several hundred moonlighting proteins thus, discovered so far play important roles. For example, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), heat-shock protein 60 (Hsp60) and tRNA synthetases play a wide range of biological roles in eukaryotic cells and some are being tested for their therapeutic potentials. While analysing the proteomics, it becomes very important to distinguish moon light proteins from those secreted contaminant proteins that may have a distinct role in cell lyses events.

Working with the fungus *Penicillium chrysogenum*, Jami *et al.* (2010b) offered some useful tips for the collection of secreted proteins *viz.*, 1) careful mycelia elimination by using nylon filters, 2) rigorous centrifugation and filtration through 0.45 μ m filters or 3) the use of low temperatures to avoid mycelial degradation. Sample collection is also crucial to discard those non-natural secreted proteins. Interestingly, Jami *et al.* (2010b) have observed increasing levels of protein in the broth even after the culture reached the stationary phase and established a linear correlation between the presence of proteins in the culture medium and biomass production. These data strongly suggests that some of the non natural proteins might have arrived to the culture medium as a result of cell lysis. Consequently, samples collection at an early time of point is recommended in accordance with the optimal moment of extracellular protein secretion. Further, the knowledge of the protein abundance at the intracellular proteome helps to clarify possible cell lysis events. For example, Shah *et al.* (2009) recorded the abundance of intracellular proteins like malate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase in case of *B. cinerea* and flavohemoglobin and manganese superoxide dismutase in case of *P. chrysogenum* during cell lysis. Although mycelial lysis is an undesirable event with respect to fungal perspective, more interestingly the *in vitro* and *in planta* secretome of *Fusarium graminearum* demonstrates the presence of 13 non-secreted proteins only under *in planta* conditions (Paper *et al.*, 2007). Some of these proteins are potent immunogens, which clearly indicates that even significant levels of fungal lysis during plant pathogenesis is mandatory or an useful event for establishing the infection by the fungal population.

New bioinformatics tools are also available to characterize the classical or non classical secreted proteins by means of prediction of secretion signal motifs (Bendtsen *et al.*, 2004 a, b). It is well known that peroxisome abundance secreted by *P. chrysogenum* can be rapidly decreased through

autophagic pathways, which could selectively degrade peroxisomes by fusion to lysosomes or vacuoles (Oku and Sakai, 2010). Thus, integration of peroxisomes into vacuoles may lead to the secretion of proteins located in the peroxisomal matrix by exocytosis, a mechanism that has been discussed by Martín *et al.* (2010) as an alternative route for the release of penicillin from peroxisomes in to culture medium.

Organelles, membrane and cell wall proteome

The interface of filamentous fungi with their local habitat and inner cell contents is mediated by the entrenched proteins that may be present in the cell wall, plasma and organelle membranes. Cell wall is referred as the largest organelle and dynamic structure of a filamentous fungus, fulfilling many vital functions, such as physical support, selective permeability barrier, osmotic stability, immobilized enzyme support, cell-cell interactions and morphogenesis (Pitarch *et al.*, 2008). Mostly, the proteomic analyses of filamentous fungi target with three important aspects *viz.*, 1) the mycelial extract (whole cell) 2) the cytosolic proteins and 3) the secretome. Even so, the membranes of organelles, like mitochondria, which includes proteins of the transport systems (e.g. porins), as well as proteins involved in fusion, fission, morphology and the inheritance of the organelle, remains unknown. Hence, mapping of organelle proteome of different cell components will be very much fascinating to reveal various aspects of fungal biology, architecture and metabolite production (like penicillin production in the microbodies of *P. chrysogenum*). Organelle enrichment and purification will be the most crucial step in the organelle proteomics of fungi (Carlos Barreiro *et al.* 2012). Homogeneous and reproducible cell disruption using automatic grinders or French press or manual methods including bead beating and crude organelle separation and enrichment have been suggested by Ferreira de Oliveira and de Graaff (2011). On the other hand enzymatic degradation for protoplast isolation followed by gentle lysis is also recommended. Additionally, methods like detergent fractionation, filtration, low-speed and ultracentrifugation, centrifugal elutriation, immunomagnetic separation can help in debris elimination and organelle isolation (like mitochondria vacuoles, microbodies, endosomes and vesicles). However, in filamentous fungi organelle isolation by cell disruption is cumbersome and very much limited due to their compact cell wall and protease production.

Moreover, the hydrophobicity of the membrane or cell wall surrounded proteins hinders isolation and analysis of such proteins. Hernández-Macedo *et al.*, (2002) used SDS-PAGE and 2D electrophoresis for plasma membrane and cell wall protein separation in cases of *P. chrysosporium* and *Lentinula edodes*. Dongbin Lim *et al.*, (2001) identified 32 protein spots by nanoelectrospray tandem mass spectrometry and reported around 220 proteins at various locations

of the cell envelope of *Trichoderma reesei*. The most abundant protein reported was HEX1, the major protein in Woronin body, suggesting that this structure unique to filamentous fungi is linked to the cell envelope. Glycosyl-phosphatidyl-inositol linked (GPI) proteins affixed to plasma membrane play a role in fungal morphogenesis and cell wall organization (Bruneau *et al.*, 2001). A total number of 10 predicted GPI-anchored proteins have been reported by de Groot *et al.* (2009) in the cell wall fractions of *A. nidulans*. Investigation of drug resistance mechanisms in fungi is yet another unexplored area. In this regard scientists are trying to fix potential biomarkers to assess the efficacy of drugs. Caspofungin is a drug used to contain *A. fumigates*. Cagas *et al.* (2011b) investigated fungal proteomic response of *A. fumigatus* to drug exposure by performing 2D electrophoresis and iTRAQ method from 4 subcellular compartment fractions *viz.*, cell wall and plasma membrane, cytoplasm, microsomes and extra cellular secretion. Microarrays were also performed and the results indicated that the cell wall proteins were often found to be highly glycosylated posing difficulties for separation from wall bound polysaccharides. Membrane proteins are highly hydrophobic and cannot be dissolved with routine procedure. Amusingly, the polarized growth of filamentous fungi requires a sequential supply of proteins and lipids to the hyphal tip. This transport is managed by vesicle trafficking via the actin and microtubule cytoskeleton. Hence, the arrangement of the cytoskeleton is a crucial step to establish and maintain the cell polarity. Norio Takeshit (2016) extensively reviewed the mechanism of polarized growth with special emphasis on the role of actin and microtubule cytoskeleton and polarity marker proteins. Rapid insertions of membranes via highly active exocytosis at hyphal tips could quickly dilute the accumulated polarity marker proteins. Transient assembly and disassembly of polarity sites at hyphal tip was revealed through high resolution microscope during the process of polarization. Priyadharshini *et al.*, (2017) investigated the function of morphogenesis related enzymes *viz.*, *mannitol dehydrogenase (MtDH)*, *xylanase*, *laccase*, *tyrosinase* and *lipoxygenase (LOX)* activities at seven different growth stages *viz.*, pinhead, tiny button, button, elongation I and II, maturation, complete maturation both in pileus and stipe of milky mushroom, *Calocybe indica* var. APK2 and CBE-TNAU-1523 wild isolate. The results indicated that the secretion level of MtDH, xylanase, laccase, tyrosinase and LOX in the mushroom reached the maximum level at stage five (elongation II) followed by stage four (elongation I) and the rate of increase in activity was higher from stage one to stage five in pileus when compared to stipe in both APK2 variety and CBE-TNAU-1523 wild strain of *Calocybe indica*. Among, all the enzymes assayed, the activity of xylanase was recorded maximum at all the seven stages of mushroom growth followed by lipoxygenase activity. The results of this study also depicts that

the lipoxygenase enzyme plays a crucial role in the production of volatile compounds which may influence the flavour and fruiting body formation of mushroom. Thus, the proteomic approach in filamentous fungi remains challenging.

Techniques in fungal proteomics

Use of high-throughput tools for complete genomic DNA sequencing, revealing of mRNA-sequences, counting of specific peptides, profiling of metabolites and secretomes enabled the modern day biologists to unravel the complex and interactive networks made out of genes and their proteins. Proteome, in the simplest sense means a set of proteins thought to be expressed by any living organism. These proteomes are based on the translation of a completely sequenced genome that may include the sequences derived from extra-chromosomal elements such as plasmids or cellular organelle. Some proteomes may also include protein sequences based on high quality cDNAs. These sequences are included by manual review of supporting evidences available from the nearest close relatives, because sometimes they cannot be mapped while assembling the genome sequences either due to sequencing errors or due to experimental gaps. Proteome is otherwise known as the protein which is produced as the compliment of the genome and individual gene could produce individual protein.

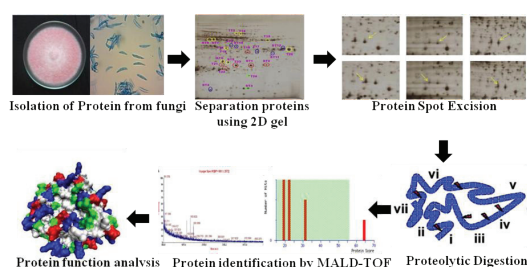


Fig.1. Flow diagram of Proteomics techniques

Bhadauria *et al.* (2007) detailed the techniques in proteomics, which include a) sample preparation, b) separation of proteins by 2-D gel electrophoresis, c) excision of peptide mixed protein spot from the gel, c) analysis of eluted protein spots by MALDI-TOF d) sequencing of peptide mass through Mass Spectrometry, e) search for corresponding gene in biological data bases with bioinformatics tools, f) knocking out specific gene by reverse genetics (or) if the protein or gene is found to be new, primer designing to get the specific cDNA and g) localization of the presence of protein in that particular host by cDNA labelling through green fluorescent protein (GFP) or genome sequence tags like GST, HIS-TAGS *etc.* Proteomic studies of plant–pathogen interactions have been revealed in several cases. Mehta *et al.* (2008) overviewed plant–pathogen interaction and indicated that plants possess receptors that can activate basal resistance, mediated by pathogen-associated molecular patterns (PAMPs) or cell wall-degrading enzymes (CWDEs), which may result in

a compatible or incompatible interaction. In both the cases, several defence-related and biotic stress-responsive proteins are induced. Suppression of plant defences by pathogen effectors leads to susceptibility in host plants. Some host plants express resistance (R) proteins, which guard against this interference and trigger a specific resistance, referred to as the hypersensitive response (HR). Dongbin Lim *et al.*, (2001) extracted and separated a total of 220 cell envelope-associated proteins from *Trichoderma reesei* mycelium. Altogether they examined 56 proteins by nanoelectrospray tandem mass spectrometry and obtained amino acid sequences for 32 protein spots, of which 20 protein spots were identified by advanced BLAST searches against all databases available. The most abundant protein found in association with the cell envelop in *T. reesei* was HEX1, the major protein known for its presence in Woronin body, a unique structure in filamentous fungi. In addition, vacuolar protease A, enolase, glyceraldehyde-3-phosphate dehydrogenase, transaldolase, protein disulfide isomerase, mitochondrial outer membrane porin, diphosphate kinase and translation elongation factor beta have also been reported. The tandem mass spectrometry also gave clear signals for proteins, but no match was found in the data bases. This result suggests that a vast majority of proteins associated with a filamentous fungal cell wall are novel.

Bioinformatics tools for fungal secretome protein analysis

The secretome has been studied in multiple fungal genomes to elucidate the potential role of those protein collections involved in a number of metabolic processes from host infection to wood degradation. The major group of secreted fungal proteins are processed by the classical secretory pathway (Lum and Min, 2011). The software Signal P 3.0 was used by Bendtsen *et al.*, (2004a) for the identification of classical secretion signal motifs presents in proteins (<http://www.cbs.dtu.dk/services/SignalP/>). This software is integrated in different bioinformatics platforms for secreted proteins identification. They also created a prediction program of secreted proteins by means of non-classical signal motifs (Bendtsen *et al.*, 2004b) and it was called Secretome P, which also has shown good results in the fungal protein analysis (<http://www.cbs.dtu.dk/services/SecretomeP/>) as referred by Shah *et al.* (2009) and Jami *et al.* (2010b). On the other hand, Phobius software (Kall *et al.*, 2004) is used to identify signal peptides and to discriminate putative transmembrane domains (<http://phobius.cgb.ki.se/index.html>). Fungal Secretome Database or the Fungal Secretome Knowledge Base (<http://fsd.snu.ac.kr/>) allow proper identification of secreted proteins by the compilation of different software (Choi *et al.*, 2010; Lum and Min, 2011). SignalP 3.0, SigCleave, SigPred, RPSP, TMHMM 2.0c, TargetP 1.1b, PSort II, SecretomeP 1.0f and predictNLS are also used to predict the presence of signal peptides or nuclear localization signals,

trans-membrane helices, protein probable location and secretion by non classical pathways. Keeping amino acid composition as key factor to recognize secretory proteins, SECRETOOL comprises a group of web tools that enable secretome predictions out of amino acid sequence profiles, up to complete fungal proteomes, in one step and is freely available at <http://genomics.cicbiogune.es/SECRETOOL/Secretool.php>. (Cortázar *et al.*, 2014).

Conclusion

To date, Omics sciences and secretome proteomics in particular, appear to be an outstanding way to decipher the enzymatic toolbox of filamentous fungi. The nature of fungal secretomes is closely dependent on their biotic and abiotic environment, a property that depends on the ecological spectrum of fungal species. The structure of these secretomes consists of hydrolases necessary to the fungal food supply. A better understanding of fungal biology and its industrial applications, plant-fungus interactions is needed one. It can be obtained by employing various methodological approaches, one of them being secretome proteomics. This technology allows qualitative and quantitative measurements of large numbers of proteins that directly influence cellular biochemistry and thus, provide accurate analysis of cellular state or system changes during growth, development and response to environmental factors. Secretome proteomics studies have firmly established the presence of a substantial level of secreted proteins lacking signal peptides and indicated a large degree of fungal species specificity in the composition of secreted proteins. Finally, we conclude that different secretome components reflect lifestyle-associated genomic adaptations in fungi. Information's from this study provide new insights into the genetic basis and molecular evolution of fungal lifestyles and also establish a solid foundation for future discovery and functional validation of secretome proteins.

References

- Barros, B.H., da Silva, S.H., dos Reis Marques Edos, R., Rosa, J.C., Yatsuda, A.P., Roberts, D.W. and Braga, G.U. 2010. A proteomic approach to identifying proteins differentially expressed in conidia and mycelium of the entomopathogenic fungus *Metarhizium acridum*. *Fungal Biol.*, **114**(7): 572-9.
- Bendtsen, J.D., Jensen, L.J., Blom, N., von Heijne, G. and Brunak, S. 2004b. Feature based prediction of non-classical and leaderless protein secretion. *Protein Eng. Des. Sel.*, **17**(4): 349-356.
- Bendtsen, J.D., Nielsen, H., von Heijne, G. and Brunak, S. 2004a. Improved prediction of signal peptides: Signal P 3.0. *J. Mol. Biol.*, **340**(4):783–795.
- Bennett, J.W. 2006. The molds of Katrina. In: The Sciences 2.0. The Molds of Katrina: Report from the Field. Dan Van Atta (ed.), New York Academy of Sciences.
- Bruneau, J.M., Magnin, T., Tagat, E., Legrand, R., Bernard, M., Diaguin, M., Fudali, C. and Latge, J.P. 2001. Proteome analysis of *Aspergillus fumigatus* identifies glycosylphosphatidylinositol-anchored proteins associated to the cell wall biosynthesis. *Electrophoresis.*, **22**(13): 2812–2823.
- Cagas, S.E., Jain, M.R., Li, H. and Perlin, D.S. 2011b. Profiling the *Aspergillus fumigatus* proteome in response to caspofungin. *Antimicrob. Agents Chemother.*, **55**(1): 146-154.
- Cao, T., Kim, Y.M., Kav, N.N.V. and Strelkov, S.E. 2009. A proteomic evaluation of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat, reveals major differences between virulent and avirulent isolates. *Proteomics*, **9**(5): 1177–1196.
- Carlos Barreiro., Carlos García-Estrada and Juan F. Martín. 2012. Proteomics methodology applied to the analysis of filamentous fungi - new trends for an impressive diverse group of organisms, tandem mass spectrometry - applications and principles, Dr Jeevan Prasain (Ed.), ISBN: 978-953-51-0141-3.
- Choi, J., Park, J., Kim, D., Jung, K., S. and Y.H. Lee. 2010. Fungal secretome database : integrated platform for annotation of fungal secretomes. *BMC Genomics.* **11**(105) : 1-15.
- Cobos, R., Barreiro, C., Mateos, R.M. and Coque, J.J.R. 2010. Cytoplasmic- and extracellular proteome analysis of *Diplodia seriata*: a phytopathogenic fungus involved in grapevine decline. *Proteome Sci.*, **8**: 46.
- Cortazar, P., Zhang, L., Untch, M., Mehta, K., Costantini, J.P., Wolmark, N., Bonnefoi, H., Cameron, D., Gianni, L., Valagussa, P., Swain, S.M., Prowell, T., Loibl, S., Wickerham, D.L., Bogaerts, J., Baselga, J., Perou, C., Blumenthal, G. Blohmer, J., Mamounas, E.P., Bergh, J., Semiglazov, V., Justice, R., Eidtmann, H., Paik, S., Piccart, M. Sridhara, R., Fasching, P.A., Slaets, L., Tang, S., Gerber, B., Geyer, C.E.Jr., Pazdur, R., Ditsch, N., Rastogi, P., Eiermann, W. and G. Von Minckwitz. 2014.
- de Groot, P.W., Brandt, B.W., Horiuchi, H., Ram, A.F., de Koster, C.G. and Klis, F.M. 2009. Comprehensive genomic analysis of cell wall genes in *Aspergillus nidulans*. *Fungal Genet Biol.*, **46**(1): 72-81.
- Dongbin Lim., Hains, P., Walsh, B., Bergquist, P. and Nevalainen, H. 2001. Proteins associated with the cell envelope of *Trichoderma reesei*: a proteomic approach. *Proteomics*, **1**(7): 899-909.
- Doyle, S. 2011. Fungal Proteomics: from identification to function. *FEMS Microbiol Lett.*, **321**(1): 1–9.
- Ellis, S.D., Boehm, M. J. and Mitchell, T. K. 2008. Fungal and Fungal-like Diseases of Plants. In: Fact sheet: Agriculture and Natural Resources. The Ohio State University. pp. 401-407.
- Espino, J.J., Gutiérrez-Sánchez, G., Brito, N., Shah, P., Orlando, R. and González, C. 2010. The *Botrytis cinerea* early secretome. *Proteomics*, **10**(16): 3020-3034.
- Fernández-Acero, F.J., Colby, T., Harzen, A., Carbu, M., Wieneke, U., Cantoral, J.M and Schmidt, J. 2010. 2-DE proteomic approach to the *Botrytis cinerea* secretome induced with different carbon sources and plant-based elicitors. *Proteomics*, **10**(12): 2270-2280.
- Ferreira de Oliveira, J.M. and de Graaff, L.H. 2011. Proteomics of industrial fungi: trends and insights for biotechnology. *Appl Microbiol Biotechnol.*, **89**(2): 225-37.
- Gao, Q., Kai Jin., Sheng-Hua Ying., Yongjun Zhang., Guohua Xiao., Yanfang Shang., Zhibing Duan., Xiao Hu., Xue-Qin Xie., Gang Zhou., Guoxiong Peng., Zhibing Luo., Wei Huang., Bing Wang., Weiguo Fang., Sibao Wang., Yi Zhong., Li-Jun Ma., Raymond J. St. Leger., Guo-Ping Zhao., Yan Pei., Ming-Guang Feng., Yuxian Xia. and Chengshu Wang. 2011. Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae*

- and *M. acridum*. *PLoS Genet.*, **7**(1): e1001264.
- Greenbaum, D., Luscombe, N.M., Jansen, R., Qian, J. and Gerstein, M. 2001. Interrelating different types of genomic data, from proteome to secretome: 'oming in on function. *Genome Res.*, **11**(9): 1463-1468.
- Hawksworth, D.L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol. Res.* **95**: 641-655.
- Hernández-Macedo, M.L., Ferraz, A., Rodríguez, J., Ottoboni, L.M. and De Mello, M.P. 2002. Iron-regulated proteins in *Phanerochaete chrysosporium* and *Lentinula edodes*: differential analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis and two-dimensional polyacrylamide gel electrophoresis profiles. *Electrophoresis*, **23**(4): 655-661.
- Jami, M.S., García-Estrada, C., Barreiro, C., Cuadrado, A.A., Salehi-Najafabadi, Z and J. F. Martín. 2010b. The Penicillium chrysogenum extracellular proteome. Conversion from a food-rotting strain to a versatile cell factory for white biotechnology. *Mol Cell Proteomics*, **9**(12): 2729-2744.
- Kall, L., Krogh, A. and E.L. Sonnhammer. 2004. A combined transmembrane topology and signal peptide prediction method. *J. Mo. L Biol.*, **338**(5) : 1027-36.
- Karkowska-Kuleta, J. and Kozik, A. 2014. Moonlighting proteins as virulence factors of pathogenic fungi, parasitic protozoa and multicellular parasites. *Mol. Oral Microbiol.* **29**: 270–283.
- Lum, G. and Min, X.J. 2011. FunSecKB: the Fungal Secretome Knowledge Base. *The journal of biological database and curation.* **11**:1-10.
- Makridakis, M. and Vlahou, A. 2010. Secretome Proteomics for discovery of cancer biomarkers. *J. Proteomics*, **73**(12): 2291-2305.
- Martín, J.F., Ullán, R.V. and García-Estrada, C. 2010. Regulation and compartmentalization of beta-lactam biosynthesis. *Microbial Biotechnol.* **3**(3): 285- 299.
- Masayuki Machida., Kiyoshi Asai., Motoaki Sano., Toshihiro Tanaka., Toshitaka Kumagai., Goro Terai., Ken-Ichi Kusumoto., Toshihide Arima., Osamu Akita., Yutaka Kashiwagi., Keietsu Abe., Katsuya Gomi., Hiroyuki Horiuchi., Katsuhiko Kitamoto., Tetsuo Kobayashi., Michio Takeuchi., David W. Denning., James E. Galagan., William C. Nierman., Jiujiang Yu., David B. Archer., Joan W. Bennett., Deepak Bhatnagar., Thomas E. Cleveland., Natalie D. Fedorova., Osamu Gotoh., Hiroshi Horikawa., Akira Hosoyama., Masayuki Ichinomiya., Rie Igarashi., Kazuhiro Iwashita., Praveen Rao Juvvadi., Masashi Kato., Yumiko Kato., Taishin Kin., Akira Kokubun., Hiroshi Maeda., Noriko Maeyama., Jun-ichi Maruyama., Hideki Nagasaki., Tasuku Nakajima., Ken Oda., Kinya Okada., Ian Paulsen., Kazutoshi Sakamoto., Toshihiko Sawano., Mikio Takahashi., Kumiko Takase., Yasunobu Terabayashi., Jennifer R. Wortman., Osamu Yamada., Youhei Yamagata., Hideharu Anazawa., Yoji Hata., Yoshinao Koide., Takashi Komori., Yasuji Koyama., Toshitaka Minetoki., Sivasundaram Suharnan., Akimitsu Tanaka., Katsumi Isono., Satoru Kuhara., Naotake Ogasawara and Hisashi Kikuchi. 2005. Genome sequencing and analysis of *Aspergillus oryzae*. *Nature*, **438**(7071): 1157–1161.
- Mehta, A., Brasileiro, A.C., Souza, D.S., Romano, E., Campos, M.A., Grossi-de-Sa, M.F., Silva, M.S., Franco, O.L., Fragoso, R.R., Bevitori, R and Rocha, T.L. 2008. Plant-pathogen interactions: what is Proteomics telling us? *FEBS J.* **275**(15): 3731-3746.
- Monteiro, V.N., do Nascimento Silva, R., Steindorff, A.S., Costa, F.T., Noronha, E.F., Ricart, C.A., de Sousa, M.V., Vainstein, M.H and Ulhoa, C.J. 2010. New Insights in *Trichoderma harzianum* Antagonism of Fungal Plant Pathogens by Secreted Protein Analysis. *Curr. Microbiol.* **61**(4): 298–305.
- Murad, A.M., Laumann, R.A., Lima Tde, A., Sarmento, R.B., Noronha, E.F., Rocha, T.L., Valadares-Ingliš, M.C and Franco, O.L. 2006. Screening of entomopathogenic *Metarhizium anisopliae* isolates and proteomic analysis of secretion synthesized in response to cowpea weevil (*Callosobruchus maculatus*) exo skeleton. *Comp Biochem Physiol C Toxicol Pharmacol.* **142**(3-4): 365-370.
- Norio Takeshita, 2016. Coordinated process of polarized growth in filamentous fungi. Bioscience, Biotechnology, and Biochemistry., <http://dx.doi.org/10.1080/09168451.2016.1179092>
- Oda, K., Kakizono, D., Yamada, O., Iefuji, H., Akita, O. and Iwashita, K. 2006. Proteomic analysis of extracellular proteins from *Aspergillus oryzae* grown under submerged and solid-state culture conditions. *Appl Environ Microbiol.* **72**(5): 3448–3457.
- Oku, M. and Sakai, Y. 2010. Peroxisomes as dynamic organelles: autophagic degradation. *FEBS J.* **277**(16): 3289-94.
- Paper, J.M., Scott-Craig, J.S., Adhikari, N.D., Cuomo, C.A. and Walton, J.D. 2007. Comparative Proteomics of extracellular proteins in vitro and in planta from the pathogenic fungus *Fusarium graminearum*. *Proteomics*, **7**(17): 3171-3183.
- Pitarch, A., Nombela, C. and Gil, C. 2008. Cell wall fractionation for yeast and fungal Proteomics. *Methods Mol Biol.* **425**: 217-239.
- Priyadarshini Bhupathi, Akkanna Subbiah Krishnamoorthy and Sivakumar Uthandi. 2017. Profiling of morphogenesis related enzymes of milky mushroom *Calocybe indica* (P & C). *Journal of Pharmacognosy and Phytochemistry.* **6**(5): 2537-2543.
- Sangeetha Chinnusamy and Akkanna Subbiah Krishna moorthy. 2017. Identification of 3'de oxyadenosine (Cordycepin) from the medicinal mushrooms, *Ophiocordyceps* spp. *International Journal of Chemical Studies.* **5**(3): 788-792
- Shah, P., Atwood, J.A., Orlando, R., El Mubarek, H., Podila, G.K. and Davis, M.R. 2009. Comparative proteomic analysis of *Botrytis cinerea* secretome. *J. Proteome Res.* **8**(3): 1123-1130.
- Suárez, M.B., Sanz, L., Chamorro, M.I., Rey, M., González, F.J., Llobell, A and Monte, E. 2005. Proteomic analysis of secreted proteins from *Trichoderma harzianum*. Identification of a fungal cell wall induced aspartic protease. *Fungal Genet Biol.* **42**(11): 924-934.
- Tjalsma, H., Bolhuis, A., Jongbloed, J.D.H., Bron, S. and van Dijk, J.M. 2000. Signal peptide dependent protein transport in *Bacillus subtilis*: a genome-based survey of the secretome. *Microbial Mol Biol Rev.* **64**(3): 515–547.
- Vijai Bhaduria., Wen Sheng Zhao., Li-Xia Wang., Yan Zhang., Jun-Hua Liu., Jun Yang., Ling-An Kong and You-Liang Peng., 2007. Advances in fungal proteomics. *Microbio. Res.*, **162**: 193-200.