We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Use of Mass Spectrometry for Characterization of Fungal Secretomes

Eduardo Callegari¹ and Mario Navarrete²

¹*Division of Basic Biomedical Sciences, Sanford School of Medicine,
University of South Dakota*

²*Facultad de Ciencias Biológicas, Universidad Andres Bello, Santiago*

¹USA

²Chile

1. Introduction

Filamentous fungi are microorganisms with a great capacity of produce and export enzymes to the extracellular media. These enzymes have been studied from different point of views and many properties have been attributed, some of them with biotechnological applications. A traditional way of studying these enzymes has been through purification, characterization and sequencing of individual enzymes. This methodology has shown, so far, remarkable properties that help to understand biological phenomena. For example, the enzyme acetyl xylan esterase II is produced by the lignocellulolytic fungus *Penicillium purpurogenum* especially when the carbon source is acetylated xylan. Under optimal conditions this enzyme was purified and sequenced (Gutierrez et al. 1998) and then its structure was elucidated from its crystal with high resolution (Ghosh et al. 2001). In a later work the expression of this enzyme was evaluated in culture supernatants coming from the fungus grown on different carbon sources where the glucose was a condition of repression for this enzyme (Chavez et al. 2004). New questions can be made for this enzyme that participates in a complex process of degradation of xylan, one of the components of hemicelluloses, which degradation is a key step for later production of chemicals and bio fuels. Since this enzyme is an isoenzyme, is it possible that more isoenzymes can be expressed, under what conditions could this be? Is it possible that more isoforms of this isoenzyme are there? Is this the only enzyme varying when the carbon source changes? What other proteins are varying? And is it possible that this enzyme is part of multienzyme complexes? These new questions arise when one realizes the complexities of the proteome. In the late nineties it was propose, arguing that it is unlikely that an enzyme is alone and that it is very possible that a protein could be interacting with ten other proteins approximately (Alberts 1998). Given the emergence of new technologies that allowed the improvement of protein separation, genome sequencing and mass spectrometry, it has been possible to address these many questions from the point of view of proteomics. Extensive has been the use of proteomics in comparative studies in biomedicine and the future of this technologies in the solution of several diseases is promising (Plymoth and Hainaut 2011). These technologies are available to be applied in several processes and lately have been used in fungi. The

many alternatives that proteomics offer makes that there is not an established protocol and that there are different answer depending of the proteomic approach.

Filamentous fungi are able to grow in different carbon sources; the ability to sense the environment and then change protein expression inside the cell and in the extracellular media, makes this system very attractive for the study and understanding of protein expression (Aro, Pakula, and Penttila 2005). In this chapter we will focus in the use of these technologies in the study of the extracellular portion of fungi called the secretome.

2. The fungal secretome

The secretome is a portion of the cellular proteome which include all secreted proteins, either anchored to cell surface or in the extracellular milieu, and the proteins involved in secretory pathway (Tjalsma et al. 2000). The protein composition of that subgroup of proteins is susceptible to stimuli from the environment that produces drastic changes in protein profile. Among the principal factors that influences protein pattern in fungal secretomes are: carbon source (Paper et al. 2007) and liquid versus solid medium (Oda et al. 2006). The characterization of the secretome is very complex, the composition and size of it will depend on the conditions of growth and the tools employed. Carbon source is able to modify the size of a secretome, for example glucose exerts a strong repression over the hemicellulases of *Aspergilli*, *Trichoderma* and *Penicillia* (Chavez, Bull, and Eyzaguirre 2006).

3. Displaying the secretome

Although the study of protein biochemistry is not a new area of research, it is proteomics; this integrates techniques of protein biochemistry introducing new techniques of protein separation and sequencing. Protein biochemistry focus on the study of structure and function of proteins involving the study of aminoacid sequence, structure determination and modeling in order to determine how structure rules the function. Biophysics and enzymologist make analysis of this kind to study single proteins, one at a time. Proteomics, on the other hand worries about the study of multiprotein systems focusing on the interaction of multiple and different proteins as part of a connected network. Analysis are directed to complex mixtures and the identification is not through the complete sequence but through partial sequences held by growing databases (Liebler 2002). The analysis of proteomes requires tools different from the employed on genetic analysis (Romiti 2006). At the present time there are several alternatives to separate the proteins from a secretome, being two-dimensional electrophoresis coupled to mass spectrometry the most widely used. An alternative to this technique is the gel free analysis using mass spectrometry. Here we will describe several cases from bibliography where the different approaches were applied and different results were obtained. A proteomic study should provide any of the following information regarding the identification of proteins, differential expression of enzymes, post-translational modifications, relative abundance and the possible association of enzymes producing high molecular weight multienzyme complexes.(Griffin, Goodlett, and Aebersold 2001).

4. The case of *Penicillium purpurogenum*

The fungus *Penicillium purpurogenum* has a great ability of adaptation to different environments. It is thought that this is due to changes in the protein profile allowing the

fungus to degrade more efficiently the hemicelluloses. An analysis of the secretome was performed using proteomics tools. The first approach was to grow the fungus in the following carbon sources: acetylated xylan, sugar beet pulp, pectin and fructose. Acetylated xylan is polyacetylated by chemical methods starting from oat spelt xylan (sigma)(Johnson et al. 1988) and it has been observed that is a powerful inducer of acetyl xylan esterase (Egana et al. 1996). Sugar beet pulp is an important agricultural residue in Chile and is composed by 50% pectin and 20% cellulose (Saulnier. and Thibault. 1999). Pectin was obtained from citrics (sigma). After four days of growth these supernatants coming from carbon sources composed of complex polysaccharides were submitted to two-dimensional electrophoresis and compared with a supernatant coming from a culture containing fructose as carbon source. The first two-dimensional electrophoresis were performed with pH 3-10 strips for the first dimension, where all the spots were located to the acidic zone of the gel, a feature observed in fungal secretomes (Bouws, Wattenberg, and Zorn 2008). After that it was decided to use pH 4-7 strips resulting in better resolution gels (Figure 1) with spots of different molecular weights ranging from 20 kDa to 200 kDa. At first sight it can be noted in the gels stained with Sypro ruby, that complex carbon sources like acetylated xylan, sugar beet pulp and pectin induce the fungus to produce and secrete a great variety of enzymes unlike fructose that produces a pattern with a few number of spots. The analysis of the spots was made by tandem mass spectrometry using a Q-TOF instrument. Experimental data produced by the instrument was submitted to MASCOT, where a search was performed in databases containing fungal genomes. Among the proteins identified there are several enzymes involved in the degradation of the substrate (Navarrete et al, submitted for publication) and other enzymes that are common for the complex polysaccharides. The effect of fructose is completely different, its behavior is very similar to the effect of catabolite repression that glucose exerts on filamentous fungi (Chavez, Bull, and Eyzaguirre 2006). It is evident that the carbon sources acetylated xylan and sugar beet pulp are very different from the point of view of the chemical composition; therefore a differential expression of hemicellulases is expected. There are some software available with the ability to compare gels and to establish differences in protein pattern like is the case of PDQuest (Bio-Rad). In two-dimensional electrophoresis is possible to observe that some spots are located very close to each other sharing molecular weight but differing pI. These spots generally correspond to the same protein with different post translational modifications (PTMs) and is usually observed in fungi as is the case for *Penicillium chrysogenum* (Jami et al. 2010). This particular phenomenon observed is due to nature of this kind of electrophoresis and to the resolution that is possible to achieve when the preparation of samples is adequate. This technique requires of good quality sample and denaturant conditions. Concentration of urea and thiourea are usually high in buffers previous to the first dimension. After isoelectric focusing, strips are submitted to two equilibration steps with buffer containing strong reducing agents like dithiotreitol (DTT) or tributylphosphine(TBP), and a second buffer containing alkylating agent usually iodoacetamide (IAA) (Simpson 2003). The combination of these chemicals produces denaturation of proteins to improve resolution. The obvious consequence is that under these conditions is not possible to measure enzyme activity and there is no possibility to know about protein-protein interactions.

Although the differences in protein expression are very clear in *Penicillium purpurogenum* grown on different carbon sources, in some cases the difference could be more difficult to appreciate and therefore other technologies must be applied. In order to make two-dimensional electrophoresis more reproducible it was developed the differential gel

Fig. 1. Differential expression of hemicellulases secreted by the fungus *Penicillium purpurogenum*. The proteins of the different supernatants were separated by two-dimensional electrophoresis. The gels were stained with Sypro ruby. Proteins were extracted from supernatants coming from cultures using acetylated xylan (A), sugar beet pulp (B), pectin (C) and fructose (D). In a first dimension proteins were submitted to an isoelectric focusing using 17 cm pH 4-7 range strips. The second dimension corresponds to SDS-PAGE of 20 x 20 cm². The spots from gels were cut and analyzed by tandem mass spectrometry.

electrophoresis, which introduces the use of the fluorescent dyes Cy2, Cy3 and Cy5. In this technique two protein samples are pre labeled, then mixed and loaded in same gel (Unlu, Morgan, and Minden 1997). This technique was applied in *Aspergillus fumigatus* to evaluate metabolic changes under hypoxia (Vodisch et al. 2011). This was also applied to *P. purpurogenum* to analyze two conditions: acetylated xylan and sugar beet pulp. It is helpful to examine one gel where the two different groups of proteins are displayed in different colors (figure 2) where green is assigned to sugar beet pulp, red to acetylated xylan and in orange to yellow are the spots present in both conditions. The analysis by mass spectrometry of the spots and DIGE allowed us to clearly establish for example that β -xylosidase is present exclusively in acetylated xylan, and that pectate lyase is specific for

Fig. 2. Differential gel electrophoresis (DIGE) of the secretome of *Penicillium purpurogenum* grown on different carbon source. Supernatant coming from acetylated xylan is labeled with Cy3 and the supernatant coming from sugar beet pulp is labeled with Cy 5, both supernatant are mixed together and loaded to the same 17 cm pH4-7 strip for isoelectric focusing. The second dimension is an SDS-PAGE of 20x20 cm². Images are acquired by a FX pro phosphor imager. Image B represents the protein pattern under acetylated xylan labeled with the chromophore Cy3, and image C is the protein pattern for sugar beet pulp secretome labeled with Cy5. The fusion of B and C result in image A. The most representative spots were excised from the gel and identified by tandem mass spectrometry (A). The most representative identities are marked in 1A.

sugar beet pulp. Mannosidases and arabinofuranosidase 2 is found in both conditions as indicated by the color. It is also possible to state that exoglucanase I present in sugar beet pulp and β -xylosidase present in acetylated xylan are submitted to post translational modifications producing the phenomenon of spot train since all of the spot in the train have the same identity.

So far we now know that the chemical composition of the carbon source produces differential expression of enzymes, and that the carbon source induces the expression of enzyme specific for the carbon source degradation. We can also assume that some post translational modifications could be occurring in this fungus like phosphorylations that

modify pI, but more studies are needed. But a proteome is even more complex and other phenomenon could be present. The modulation of enzyme activity could be affected by protein-protein interaction for enzymes involved in xylan degradation, such is the case of *Fibrobacter succinogenes* where a xylanase is able to interact with an acetyl xylan esterase. This interaction generates synergy between these two activities (Kam et al. 2005). For another acetyl xylan esterase in *Termitomyces clypeatus*, it has been shown that the interaction with other enzymes provide a higher resistance to chemical denaturing agents than the resistance that offer the enzyme alone (Mukhopadhyay et al. 2003).

The most studied example of protein interaction involved in cellulose degradation is the cellulosome. These are structures formed by different proteins and have the ability to degrade cellulose and also hemicellulose due to the presence of all of the necessary enzymes. Their main characteristic is the presence of scaffolding proteins, which have been sequenced and very well studied, whose function is to keep the protein structure united (Ding et al. 1999).

So, this demonstrates that interactions are in direct benefit of the producer fungus. This experimental evidence along with the fact that hemicelluloses are chemically complex needing several enzyme activities acting on the same substrates makes suitable the idea that in fungi could be more than two proteins interacting. An analysis of the secretome of *P. purpurogenum* was performed using blue native (Wittig, Braun, and Schagger 2006). In this technique proteins are submitted to a gel in native conditions without interrupting protein interactions. In this case the samples used for this kind of analysis were culture supernatants coming from acetylated xylan, sugar beet pulp, corn cob and glucose. The SDS is replaced by Coomassie blue G-250 that is able to add negative charges to the proteins without denature the protein sample. Under these conditions it is possible to do a zymogram over the gel, in this case we used methyl umbelliferyl acetate as substrate to evaluate acetyl esterase activities (Figure 3.I). This evidence shows that high molecular weight proteins are responsible for the activities, and according to two-dimensional electrophoresis in figure 1, the highest protein is about 200 kDa. In figure 3 protein bands with enzyme activities are over 250 kDa and are present in the three complex polysaccharides. In this case it is evident the catabolite repression that glucose exerts.

The pattern in figure 3.I shows that active high molecular weight complexes are present in the different conditions in changing molecular weights. This could be indicating that the complexes could be integrated by different subunits with different activities. This was evaluated by the use of antibodies that were used to co-immune precipitate the different complexes and by western identify subunits (Gonzalez-Vogel et al. 2011). A purification of one complex was performed by the use of different columns until a pure complex was obtained (Figure 3.II). The complex was submitted to SDS-PAGE and the subunits were identified by mass spectrometry. This approach has been used to analyze protein complexes in bacteria like *Escherichia coli* (Lasserre et al. 2006) and *Helicobacter pylori* (Pyndiah et al. 2007).

The discovery of protein complexes in *P. purpurogenum* harboring all of the necessary enzyme to degrade hemicellulose brings another point of view for the understanding of the complex process of degradation of lignocellulose by filamentous fungus. The interactions between subunits are strong and specific since they were not affected by treatment with high concentration of ammonium sulfate (Gonzalez-Vogel et al. 2011) still, new questions arise. The interactions could be mediated by direct protein-protein interactions, by the glycosylations, might be mediated by extracellular polysaccharides (Iwashita 2002) by

Fig. 3. Analysis of the secretome of *Penicillium purpurogenum* using Blue Native. Supernatants coming from cultures on different carbon sources were separated in native gels where acetyl esterase activity was measured (I,1) and the total protein pattern was stained with Sypro ruby (I,2). Lanes in I are: Molecular weight standard (M), supernatants coming from glucose (A), sugar beet pulp (B), corn cob (C) and acetylated xylan (D). Blue native of a pure complex from culture coming on sugar beet pulp. Sypro ruby staining indicates the purity of the fraction in the left lane, then in the right lane the zymogram indicates that is catalytically active. By SDS-PAGE the subunits A, B, C, D, E and F were separated and then identified by tandem mass spectrometry.

scaffolding proteins like dockerines in cellulosomes (Levasseur et al. 2004) or by core proteins (Bayer et al. 1998). All of these possibilities can be investigated by the point of view of proteomics that help to understand the fine mechanism of regulation in eukaryotes.

5. Analysis of glycosylations

Figures 1 and 2 show that is evident that post translational modifications are occurring in *P. purpurogenum* and also is evident that happens in other fungi (Bouws, Wattenberg, and Zorn 2008). This matter is very difficult to confront since the complexity of multienzyme systems is already difficult. Nevertheless, post translational modifications have been detected in cellulases of *Trichoderma reesei* (Fryksdale et al. 2002). In this study the enzymes were deglycosylated previous to two-dimensional electrophoresis; this made changes in protein pattern and also improved protein identification by mass spectrometry, because large glycosylation block trypsin.

Fig. 4. Analysis of glycosylations in the secretome of *P. purpurogenum* under catabolite repression. The supernatant coming from glucose was loaded in lane 3. In lane 2 the same supernatant was treated with PNGase F. The gel was stained with Sypro ruby and the bands were identified by mass spectrometry. The identities are in table 1.

Band n°	Protein	Microorganism	GI number	Mowse score
1	FAD dependant oxygenase	<i>Aspergillus flavus</i>	238491442	120
2	FAD dependant oxygenase	<i>Aspergillus flavus</i>	238491442	129
3	FAD dependant oxygenase	<i>Aspergillus flavus</i>	238491442	152
4	PNGase F	<i>Flavobacterium Meningosepticum</i>	157833480	643

Table 1. List of proteins identified coming from the bands in figure 4.

In the case of *P. purpurogenum* we have started to deal with this problem. We have taken the sample of protein coming from a culture grown on glucose. Glucose exerts a strong catabolite repression producing a particular protein pattern in SDS-PAGE (Figure 4, lane 3). Only one major band is detected, this makes it suitable for the study of PTMs because of the simplicity of this secretome. The secretome was treated with PNGase F and the result is observed in figure 4, lane 2. The major band is not present but a smearing. All bands were identified by mass spectrometry; bands 1, 2, and 3 are the same protein, demonstrating that the difference in molecular weight is due to glycosylation. In this case, no glycosylation was sequenced by mass spectrometry, but by the use of mass spectrometry coupled to SDS-PAGE we were able to identify the same protein suffering molecular weight changes due to treatment with PNGase. This evidence is the first step for glycosylation occurring in this fungus, and is probably occurring in other carbon sources as well.

6. Results in other fungi

Studies on ascomycetes’ secretomes identifying enzymes related to cellulose and hemicellulose degradation have been reported. Medina et al. (2004) analyzed the secretome of *Aspergillus flavus*. Utilizing 2D electrophoresis and MALDI-TOF mass spectrometry the authors attempted to identify the enzymes secreted by the fungus by comparing a rich medium (potato dextrose) with rutin (a glycoside) as sole carbon sources. They were able to identify a rather low number of spots (22 out of more than 100) from the gel (Medina, Kiernan, and Francisco 2004). Limiting factors in the identification were the lack of the genome sequence of this fungus, the presence of glycosylation (a common occurrence in extracellular fungal enzymes; (Peberdy 1994))which affects detection by MALDI-TOF, or the lack of similar sequences from other organisms in the databases. In a later publication (Medina et al. 2005), by incorporating the nanoLC-MS/MS technique, which does not have the limitations found in MALDI-TOF, they were able to identify 51 proteins. The extracellular proteome of *Aspergillus oryzae* was analyzed by comparing fungal cultures in liquid and solid media utilizing wheat bran as carbon source. By means of 1D and 2D electrophoresis and MALDI-TOF, plus the availability of expressed sequence tags from the sequenced genome, they were able to identify 29 proteins analyzing 85 spots from the solid culture and 110 from the liquid culture. An important finding is the difference in expression of proteins when comparing both media: in solid cultures the fungus secretes more protein and a larger variety of them(Oda et al. 2006).

Differences have also been found in the secretome when comparing solid and liquid cultures of *Aspergillus oryzae* grown on ground wheat (Te Biesebeke et al. 2006).

The secretome of some basidiomycetes active in lignocellulose biodegradation has also been reported. The secretome of the white-rot fungus *Phanerochaete chrysosporium* was grown on cellulose and have been compared with the open reading frames obtained from the sequenced genome. By means of liquid chromatography coupled with in-tandem mass spectrometry they have identified 32 glycosyl hydrolases out of 166 predicted, suggesting that probably many of these enzymes are expressed under different culture conditions (Wymelenberg et al. 2005). More recently, the same group (Vanden Wymelenberg et al. 2009) has extended this work reporting a transcriptome and secretome analyses of *P. chrysosporium* grown under defined ligninolytic and cellulolytic conditions so as to have a more complete view of gene expression. A total of 545 genes could be identified; however, 190 up regulated genes were predicted to encode proteins of unknown function, requiring further biochemical work for their proper identification. Additional work on the secretome of this fungus has been performed (Abbas et al. 2005) and (Ravalason et al. 2008). The first group used red oak wood chips as carbon source and could identify 16 proteins from 2D gels by LC/MS/MS sequencing, most of them acting on cellulose and hemicelluloses. Ravalason et al. (2008) grew the fungus on black pine (softwood) wood chips and identified seven glycosyl hydrolases.

The genome of the brown-rot fungus *Postia placenta* was analyzed and compared to that of the white-rot *P. chrysosporium*. Considerable differences were registered, indicating a much lower number of genes of cellulases in the brown-rot. White rot fungi degrade all components of the plant cell walls, while brown-rot can modify lignin but have a limited capacity to hydrolyze the polysaccharides of lignocellulose and have developed novel mechanisms for their degradation. An analysis of the secretome of *P. placenta* grown on aspen wood, cellulose or cotton shows, among other enzymes, the presence of various hemicellulases (Martinez et al. 2009).

The state of the art on fungal secretome studies from both ascomycetes and basidiomycetes was reviewed (Bouws, Wattenberg, and Zorn 2008). They stress the fact that many more sequenced genomes and secretome analyses are necessary for a full understanding of the strategies used by fungi in extracellular biocatalysis and for a more efficient utilization of biomass in industrial applications. Although the study of fungal secretomes is increasing, many of the reports are still of a rather preliminary nature. They tend to be descriptive with still a limited discussion of the biological significance of the findings. This is an emerging area of research, which has to overcome certain limitations. This is due in part to the still low availability of sequenced and annotated fungal genomes and to a lack of knowledge of the function of many of the proteins deduced from the genome's open reading frames, since biochemical studies of these proteins lags far behind. However, despite of these limitations, a study of the proteome (and secretome) is essential for the understanding of numerous biological functions at the molecular level.

7. Conclusion

Filamentous fungi possess a very complex and fine mechanism of regulation. The application of proteomic methods can expose the consequences of these regulation. Through the optic of the fungus *Penicillium purpurogenum*, we have seen all of the possible mechanism by separating and identifying enzymes. In present time the number of proteomic studies

involving filamentous fungi are low but increasing. Comprehensive proteomic studies should consider PTMs and protein-protein interactions since these modifications affect the behavior of multienzyme systems. Two-dimensional electrophoresis is a powerful tool that allows to display the secretome where is possible to see the evidence of post translational modifications, but, by the other hand gel free analysis allows a more wide identification of proteins including those that do not enter in gels and the ones in low quantities.

The evidence presented here show that a combination of both a approaches should be use in order to get a wider picture of the secretome.

8. References

- Abbas, A., H. Koc, F. Liu, and M. Tien. 2005. "Fungal degradation of wood: initial proteomic analysis of extracellular proteins of *Phanerochaete chrysosporium* grown on oak substrate." *Curr Genet* no. 47 (1):49-56. doi: 10.1007/s00294-004-0550-4.
- Alberts, B. 1998. "The cell as a collection of protein machines: preparing the next generation of molecular biologists." *Cell* no. 92 (3):291-4. doi: S0092-8674(00)80922-8 [pii].
- Aro, N., T. Pakula, and M. Penttila. 2005. "Transcriptional regulation of plant cell wall degradation by filamentous fungi." *FEMS Microbiol Rev* no. 29 (4):719-39. doi: S0168-6445(04)00092-0 [pii] 10.1016/j.femsre.2004.11.006.
- Bayer, E. A., H. Chanzy, R. Lamed, and Y. Shoham. 1998. "Cellulose, cellulases and cellosomes." *Curr Opin Struct Biol* no. 8 (5):548-57. doi: S0959-440X(98)80143-7 [pii].
- Bouws, H., A. Wattenberg, and H. Zorn. 2008. "Fungal secretomes--nature's toolbox for white biotechnology." *Appl Microbiol Biotechnol* no. 80 (3):381-8. doi: 10.1007/s00253-008-1572-5.
- Chavez, R., P. Bull, and J. Eyzaguirre. 2006. "The xylanolytic enzyme system from the genus *Penicillium*." *J Biotechnol* no. 123 (4):413-33. doi: S0168-1656(06)00082-4 [pii] 10.1016/j.jbiotec.2005.12.036.
- Chavez, R., K. Schachter, C. Navarro, A. Peirano, P. Bull, and J. Eyzaguirre. 2004. "The acetyl xylan esterase II gene from *Penicillium purpurogenum* is differentially expressed in several carbon sources, and tightly regulated by pH." *Biol Res* no. 37 (1):107-13.
- Ding, S. Y., E. A. Bayer, D. Steiner, Y. Shoham, and R. Lamed. 1999. "A novel cellosomal scaffoldin from *Acetivibrio cellulolyticus* that contains a family 9 glycosyl hydrolase." *J Bacteriol* no. 181 (21):6720-9.
- Egana, L., R. Gutierrez, V. Caputo, A. Peirano, J. Steiner, and J. Eyzaguirre. 1996. "Purification and characterization of two acetyl xylan esterases from *Penicillium purpurogenum*." *Biotechnol Appl Biochem* no. 24 (Pt 1):33-9.
- Fryksdale, B. G., P. T. Jedrzejewski, D. L. Wong, A. L. Gaertner, and B. S. Miller. 2002. "Impact of deglycosylation methods on two-dimensional gel electrophoresis and matrix assisted laser desorption/ionization-time of flight-mass spectrometry for proteomic analysis." *Electrophoresis* no. 23 (14):2184-93. doi: 10.1002/1522-2683(200207)23:14<2184::AID-ELPS2184>3.0.CO;2-1.
- Ghosh, D., M. Sawicki, P. Lala, M. Erman, W. Pangborn, J. Eyzaguirre, R. Gutierrez, H. Jornvall, and D. J. Thiel. 2001. "Multiple conformations of catalytic serine and histidine in acetylxylan esterase at 0.90 Å." *J Biol Chem* no. 276 (14):11159-66. doi: 10.1074/jbc.M008831200 [pii].

- Gonzalez-Vogel, A., J. Eyzaguirre, G. Oleas, E. Callegari, and M. Navarrete. 2011. "Proteomic analysis in non-denaturing condition of the secretome reveals the presence of multienzyme complexes in *Penicillium purpurogenum*." *Appl Microbiol Biotechnol* no. 89 (1):145-55. doi: 10.1007/s00253-010-2953-0.
- Griffin, T. J., D. R. Goodlett, and R. Aebersold. 2001. "Advances in proteome analysis by mass spectrometry." *Curr Opin Biotechnol* no. 12 (6):607-12. doi: S0958-1669(01)00268-3 [pii].
- Gutierrez, R., E. Cederlund, L. Hjelmqvist, A. Peirano, F. Herrera, D. Ghosh, W. Duax, H. Jornvall, and J. Eyzaguirre. 1998. "Acetyl xylan esterase II from *Penicillium purpurogenum* is similar to an esterase from *Trichoderma reesei* but lacks a cellulose binding domain." *FEBS Lett* no. 423 (1):35-8. doi: S0014-5793(98)00055-6 [pii].
- Iwashita, K. 2002. "Recent studies of protein secretion by filamentous fungi." *J Biosci Bioeng* no. 94 (6):530-5. doi: S1389-1723(02)80191-8 [pii].
- Jami, M. S., C. Garcia-Estrada, C. Barreiro, A. A. Cuadrado, Z. Salehi-Najafabadi, and J. F. Martin. 2010. "The *Penicillium chrysogenum* extracellular proteome. Conversion from a food-rotting strain to a versatile cell factory for white biotechnology." *Mol Cell Proteomics* no. 9 (12):2729-44. doi: 10.1074/mcp.M110.001412.
- Johnson, K.G., B.A. Harrisona, H. Schneidera, C.R. MacKenziea, and J.D. Fontanaa. 1988. "Xylan-hydrolysing enzymes from *Streptomyces* spp." *Enzyme and Microbial Technology* no. 10 (7):403-409 doi: 10.1016/0141-0229(88)90034-8.
- Kam, D. K., H. S. Jun, J. K. Ha, G. D. Inglis, and C. W. Forsberg. 2005. "Characteristics of adjacent family 6 acetylxylan esterases from *Fibrobacter succinogenes* and the interaction with the Xyn10E xylanase in hydrolysis of acetylated xylan." *Can J Microbiol* no. 51 (10):821-32. doi: 10.1139/w05-074.
- Lasserre, J. P., E. Beyne, S. Pyndiah, D. Lapaillerie, S. Claverol, and M. Bonneu. 2006. "A complexomic study of *Escherichia coli* using two-dimensional blue native/SDS polyacrylamide gel electrophoresis." *Electrophoresis* no. 27 (16):3306-21. doi: 10.1002/elps.200500912.
- Levasseur, A., S. Pages, H. P. Fierobe, D. Navarro, P. Punt, J. P. Belaich, M. Asther, and E. Record. 2004. "Design and production in *Aspergillus niger* of a chimeric protein associating a fungal feruloyl esterase and a clostridial dockerin domain." *Appl Environ Microbiol* no. 70 (12):6984-91. doi: 10.1128/AEM.70.12.6984-6991.2004.
- Liebler, Daniel C. 2002. *Introduction to Proteomics, tools for the new biology*. Totowa, New Jersey.: Humana Press.
- Martinez, D., J. Challacombe, I. Morgenstern, D. Hibbett, M. Schmoll, C. P. Kubicek, P. Ferreira, F. J. Ruiz-Duenas, A. T. Martinez, P. Kersten, K. E. Hammel, A. Vanden Wymelenberg, J. Gaskell, E. Lindquist, G. Sabat, S. S. Bondurant, L. F. Larrondo, P. Canessa, R. Vicuna, J. Yadav, H. Doddapaneni, V. Subramanian, A. G. Pisabarro, J. L. Lavin, J. A. Oguiza, E. Master, B. Henrissat, P. M. Coutinho, P. Harris, J. K. Magnuson, S. E. Baker, K. Bruno, W. Kenealy, P. J. Hoegger, U. Kues, P. Ramaiya, S. Lucas, A. Salamov, H. Shapiro, H. Tu, C. L. Chee, M. Misra, G. Xie, S. Teter, D. Yaver, T. James, M. Mokrejs, M. Pospisek, I. V. Grigoriev, T. Brettin, D. Rokhsar, R. Berka, and D. Cullen. 2009. "Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose

- conversion." *Proc Natl Acad Sci U S A* no. 106 (6):1954-9. doi: 0809575106 [pii] 10.1073/pnas.0809575106.
- Medina, M. L., P. A. Haynes, L. Breci, and W. A. Francisco. 2005. "Analysis of secreted proteins from *Aspergillus flavus*." *Proteomics* no. 5 (12):3153-61. doi: 10.1002/pmic.200401136.
- Medina, M. L., U. A. Kiernan, and W. A. Francisco. 2004. "Proteomic analysis of rutin-induced secreted proteins from *Aspergillus flavus*." *Fungal Genet Biol* no. 41 (3):327-35. doi: 10.1016/j.fgb.2003.11.014 S108718450300210X [pii].
- Mukhopadhyay, A., P. P. Hazra, T. Sengupta, R. Saha, R. Nandi, and S. Sengupta. 2003. "Protein-protein interaction conferring stability to an extracellular acetyl (xylan) esterase produced by *Termitomyces clypeatus*." *Biotechnol Prog* no. 19 (3):720-6. doi: 10.1021/bp0201307.
- Oda, K., D. Kakizono, O. Yamada, H. Iefuji, O. Akita, and K. Iwashita. 2006. "Proteomic analysis of extracellular proteins from *Aspergillus oryzae* grown under submerged and solid-state culture conditions." *Appl Environ Microbiol* no. 72 (5):3448-57. doi: 72/5/3448 [pii] 10.1128/AEM.72.5.3448-3457.2006.
- Paper, J. M., J. S. Scott-Craig, N. D. Adhikari, C. A. Cuomo, and J. D. Walton. 2007. "Comparative proteomics of extracellular proteins in vitro and in planta from the pathogenic fungus *Fusarium graminearum*." *Proteomics* no. 7 (17):3171-83. doi: 10.1002/pmic.200700184.
- Peberdy, J. F. 1994. "Protein secretion in filamentous fungi--trying to understand a highly productive black box." *Trends Biotechnol* no. 12 (2):50-7.
- Plymoth, A., and P. Hainaut. 2011. "Proteomics beyond proteomics: toward clinical applications." *Curr Opin Oncol* no. 23 (1):77-82. doi: 10.1097/CCO.0b013e32834179c1.
- Pyndiah, S., J. P. Lasserre, A. Menard, S. Claverol, V. Prouzet-Mauleon, F. Megraud, F. Zerbib, and M. Bonneu. 2007. "Two-dimensional blue native/SDS gel electrophoresis of multiprotein complexes from *Helicobacter pylori*." *Mol Cell Proteomics* no. 6 (2):193-206. doi: M600363-MCP200 [pii] 10.1074/mcp.M600363-MCP200.
- Ravalason, H., G. Jan, D. Molle, M. Pasco, P. M. Coutinho, C. Lapierre, B. Pollet, F. Bertaud, M. Petit-Conil, S. Grisel, J. C. Sigoillot, M. Asther, and I. Herpoel-Gimbert. 2008. "Secretome analysis of *Phanerochaete chrysosporium* strain CIRM-BRFM41 grown on softwood." *Appl Microbiol Biotechnol* no. 80 (4):719-33. doi: 10.1007/s00253-008-1596-x.
- Romiti, Monica. 2006. "Entrez Help." In, ed NCBI.
- Saulnier., Luc, and Jean-Franoise Thibault. 1999. "Ferulic acid and diferulic acids as components of sugar-beet pectins and maize bran heteroxylans." *Journal of the science of food and agriculture* no. 79:396-402.
- Simpson, Richard J. 2003. *Proteins and Proteomics: a laboratory manual*. New York: Cold Spring Harbor Laboratory Press.
- Te Biesebeke, R., A. Boussier, N. van Biezen, C. A. van den Hondel, and P. J. Punt. 2006. "Identification of secreted proteins of *Aspergillus oryzae* associated with growth on solid cereal substrates." *J Biotechnol* no. 121 (4):482-5. doi: S0168-1656(05)00550-X [pii] 10.1016/j.jbiotec.2005.08.028.

- Tjalsma, H., A. Bolhuis, J. D. Jongbloed, S. Bron, and J. M. van Dijk. 2000. "Signal peptide-dependent protein transport in *Bacillus subtilis*: a genome-based survey of the secretome." *Microbiol Mol Biol Rev* no. 64 (3):515-47.
- Unlu, M., M. E. Morgan, and J. S. Minden. 1997. "Difference gel electrophoresis: a single gel method for detecting changes in protein extracts." *Electrophoresis* no. 18 (11):2071-7. doi: 10.1002/elps.1150181133.
- Vanden Wymelenberg, A., J. Gaskell, M. Mozuch, P. Kersten, G. Sabat, D. Martinez, and D. Cullen. 2009. "Transcriptome and secretome analyses of *Phanerochaete chrysosporium* reveal complex patterns of gene expression." *Appl Environ Microbiol* no. 75 (12):4058-68. doi: AEM.00314-09 [pii] 10.1128/AEM.00314-09.
- Vodisch, M., K. Scherlach, R. Winkler, C. Hertweck, H. P. Braun, M. Roth, H. Haas, E. R. Werner, A. A. Brakhage, and O. Kniemeyer. 2011. "Analysis of the *Aspergillus fumigatus* proteome reveals metabolic changes and the activation of the pseurotin A biosynthesis gene cluster in response to hypoxia." *J Proteome Res* no. 10 (5):2508-24. doi: 10.1021/pr1012812.
- Wittig, I., H. P. Braun, and H. Schagger. 2006. "Blue native PAGE." *Nat Protoc* no. 1 (1):418-28. doi: nprot.2006.62 [pii] 10.1038/nprot.2006.62.
- Wymelenberg, A. V., G. Sabat, D. Martinez, A. S. Rajangam, T. T. Teeri, J. Gaskell, P. J. Kersten, and D. Cullen. 2005. "The *Phanerochaete chrysosporium* secretome: database predictions and initial mass spectrometry peptide identifications in cellulose-grown medium." *J Biotechnol* no. 118 (1):17-34. doi: S0168-1656(05)00152-5 [pii] 10.1016/j.jbiotec.2005.03.010.

IntechOpen

Tandem Mass Spectrometry - Applications and Principles

Edited by Dr Jeevan Prasain

ISBN 978-953-51-0141-3

Hard cover, 794 pages

Publisher InTech

Published online 29, February, 2012

Published in print edition February, 2012

Tandem Mass Spectrometry - Applications and Principles presents comprehensive coverage of theory, instrumentation and major applications of tandem mass spectrometry. The areas covered range from the analysis of drug metabolites, proteins and complex lipids to clinical diagnosis. This book serves multiple groups of audiences; professional (academic and industry), graduate students and general readers interested in the use of modern mass spectrometry in solving critical questions of chemical and biological sciences.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Eduardo Callegari and Mario Navarrete (2012). The Use of Mass Spectrometry for Characterization of Fungal Secretomes, Tandem Mass Spectrometry - Applications and Principles, Dr Jeevan Prasain (Ed.), ISBN: 978-953-51-0141-3, InTech, Available from: <http://www.intechopen.com/books/tandem-mass-spectrometry-applications-and-principles/the-use-of-mass-spectrometry-for-characterization-of-fungal-secretomes>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen