Contribution of Proteomics to the Study of Plant Pathogenic Fungi

ARTICLE *in* JOURNAL OF PROTEOME RESEARCH · NOVEMBER 2011

Impact Factor: 4.25 · DOI: 10.1021/pr200873p · Source: PubMed

2 AUTHORS:

Raquel González-Fernández
Universidad Autónoma de Ciudad Juárez
22 PUBLICATIONS **177** CITATIONS
SEE PROFILE

Jesús V Jorrin-Novo
University of Cordoba (Spain)
150 PUBLICATIONS **2,907** CITATIONS
SEE PROFILE

Journal of proteome research

REVIEWS

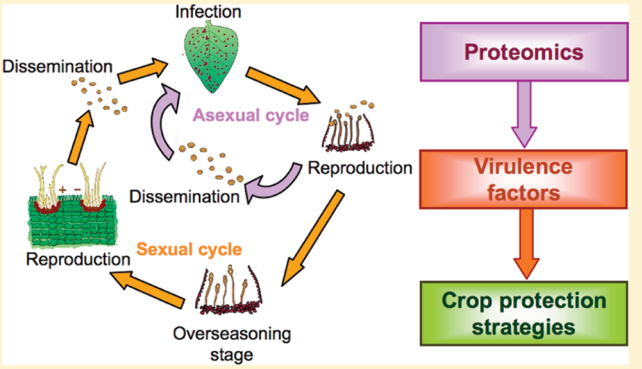
pubs.acs.org/jpr

Contribution of Proteomics to the Study of Plant Pathogenic Fungi

Raquel Gonzalez-Fernandez* and Jesus V. Jorrin-Novo

Agroforestry and Plant Biochemistry and Proteomics Research Group, Department of Biochemistry and Molecular Biology, University of Cordoba, Agrifood Campus of International Excellence, ceiA3, 14071 Cordoba, Spain

ABSTRACT: Phytopathogenic fungi are one of the most damaging plant parasitic organisms, and can cause serious diseases and important yield losses in crops. The study of the biology of these microorganisms and the interaction with their hosts has experienced great advances in recent years due to the development of moderm, holistic and high-throughput -omic techniques, together with the increasing number of genome sequencing projects and the development of mutants and reverse genetics tools. We highlight among these -omic techniques the importance of proteomics, which has become a relevant tool in plant−fungus pathosystem research. Proteomics intends to identify gene products with a key role in pathogenicity and virulence. These studies would help in the search of key protein targets and in the development of agrochemicals, which may open new ways for crop disease diagnosis and protection. In this review, we made an overview on the contribution of proteomics to the knowledge of life cycle, infection mechanisms, and virulence of the plant pathogenic fungi. Data from current, innovative literature, according to both methodological and experimental systems, were summarized and discussed. Specific sections were devoted to the most studied fungal phytopathogens: *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Fusarium graminearum*.



KEYWORDS: plant pathogenic fungi, fungal proteomics, fungal secretome, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Fusarium graminearum*

1. INTRODUCTION: PLANT PATHOGENIC FUNGI

Fungi form a large, heterogeneous eukaryotic group of living organisms. Although most fungal species are saprophytes, a number of them are animals or plants parasites, because they need a host to complete their biological cycle. Only a limited number of fungal species are able to penetrate and invade host tissues, avoiding recognition and plant defense responses in order to obtain nutrients from them, and they cause diseases which may entail sometimes the death of the host.[1] Plant pathogenic fungi have a high versatility in their infection mechanisms because different hosts and plant tissues (e.g., leaves, flowers, roots, fruits) may be affected (Table 1).

Fungal pathogens have complicated life cycles because they have both asexual and sexual reproduction, together with stages which involve the formation of different infective, vegetative, and reproductive structures.[2] The plant−fungus interaction depends on mutual recognition. Moreover, this interaction depends on the expression of pathogenicity and virulence factors in the fungus, while in the plant, it depends on the existence of passive, preformed, or inducible defense mechanisms. A number of fungal mechanisms and molecules have been shown to contribute to fungal pathogenicity or virulence, including cell wall degrading proteins,[3] inhibitory proteins,[4] and enzymes involved in the toxin synthesis.[5−8] Knowledge of the pathogenic cycle and virulence factors is crucial for the design of effective crop protection strategies, including the development of resistant plant genotypes through classical plant breeding[9] or genetic engineering,[10] fungicides,[11] or the use of biological control strategies.[12]

Fungal pathogens have an enormous impact on plant production worldwide,[13] so the strategies they use to infect plants and cause diseases demand a great attention. Pathogenicity genes have been defined as those necessary for disease development, but not essential for the pathogen to complete its life cycle *in vitro*.[14] The study of these gene products is of great relevance as potential fungicide targets for disease control, which may be discovered by the analysis of genome sequences, transcriptomes, and proteomes. Recently, a Patogen-Host Interactions database has been created (PHI-base, http://www.phi-base.org).[15] This database catalogues experimentally verified pathogenicity, virulence, and effector genes from fungal, oomycetes, and bacterial pathogens, which infect animal, plant, fungal, and insect hosts.

Studies on fungal pathogens and their interactions with plants have been performed using both classical genetics, cell biology, and biochemistry, as well as the modern, holistic, and high-throughput -omic techniques.[14,16−18] Furthermore, specific bioinformatic tools have been developed, for example, to predict

Special Issue: Microbial and Plant Proteomics

Received: September 1, 2011
Published: November 16, 2011

Table 1. Hosts and Diseases Caused by Plant Pathogenic Fungi Cited in the Text

fungus	hosts	disease
Aspergillus spp.	Corn, peanuts, cotton	Bread mold and seed decay
Blumeria graminis f.sp. hordei	Cereals and grasses	Powdery mildew
Botrytis cinerea	Different types of plants (grapevine, tomato, strawberry)	Gray mold rot
Curvularia lunata	Grasses	Leaf spot
Fusarium graminearum	Grain crops	Stem rots and seed infections
Leptosphaeria maculans	Cabbage	Black leg and foot rot
Magnaporthe grisea	Rice	Rice blast
Phakospora pachyrhizi	Soybean	Rust
Phytophthora infestans	Different types of plants (potato, carrot)	Late blight
Phytophthora palmivora	Tropical crops (coconuts, betel nuts)	Fruit rot
Phytophthora ramorum	Different types of plants (oak)	Sudden death
Pyrenophora tritici-repentis	Cereals and grasses	Leaf spot
Rhizoctonia solani	Different types of plants (cereals, tomato, cabbage)	Damping-off and fruit rots
Sclerotinia sclerotiorum	Different types of plants (canola, rice)	White mold
Serpula lacrymans	Conifers	Brown rot
Stagnospora nodorum	Wheat	Leaf spot
Thielaviopsis basicola	Conifers and herbaceous plants	Black root rot
Uromyces appendiculatus	Beans	Rust
Ustilago maydis	Corn	Smut
Verticillium dahliae	Different types of plants (tomato, olive)	Vascular wilt

gene products involved in plant−fungus pathogen interactions.[19] In recent years, the study of fungal plant pathogens has been greatly promoted by the availability of their genomic sequences and the resources for functional genomic analysis, including transcriptomics, proteomics, and metabolomics.[20] These approaches, in combination with targeted mutagenesis or transgenic studies, are unravelling molecular host−pathogen crosstalks, the complex mechanisms involving pathogenesis and host avoidance.[21]

2. PROTEOMIC RESEARCH IN PLANT PATHOGENIC FUNGI

Similar to genomics and transcriptomics, proteomics has evolved to incorporate high-throughput techniques and protocols that allow a faster analysis of large numbers of proteins.[22,23] Proteomics offers the possibility of studying simultaneously the total set of protein species that is present in a biological unit (from subcellular organelles to ecosystems), together with its cataloguing (descriptive proteomics), its abundance (quantitative proteomics), genotype-dependent variations (population proteomics), implication in development and enviromental responses changes (differential or comparative proteomics), post-translational modifications (PTMs), and interactions with other proteins and molecular entities (interactomics). By using proteomics, we try to find out how, where, when, and what for are the several hundred thousands of individual protein species in a living organism produced, how they interact with one another in order to fill the developmental program and responses to their environment. Fungal proteomic research has increased dramatically over the last years, because of the increasing number of fungal genomes sequenced. Currently, over 50 pathogenic fungal genomes have been sequenced, 20 of which are phytopathogens (Broad Institute Database, http://broadinstitute.org/science/project/fungal-genomeinitiative; Phytopathogenic Fungi and Oomycete EST Database, COGEME, http://cogeme.ex.ac.uk). Proteomics has become an integral component of all large-scale -omic and systems approaches to understand the huge complexity of fungal biochemistry.[24] Proteomic studies have been carried out in order to understand plant−fungus interactions, fungal pathogenicity, and virulence.[2,16,25−27] Further, some fungal species have attracted an increasing interest in biotechnological industry, timber trade, food science, or agriculture as biocontrol agents.[28−30]

The aim of this review is to describe and discuss the use of proteomics as a tool in the study of plant pathogenic fungus biology, and to identify relevant protein species in attack, invasion, and colonization of plant. We make a specific reference to the three most studied fungi at the proteomic level: *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Fusarium graminearum*.

The first studies using a proteomic approach were carried out in order to understand plant−fungus interactions throught the search of resistance-related proteins. The pioneer work was performed on the tomato−*Cladosporium fulvum* interaction, which allowed the characterization of the first avirulence gene product (Avr9), obtained after the purification of apoplastic fluids from susceptible plants. Xylem proteins were resolved by preparative polyacrylamide gel electrophoresis followed by reverse-phase high-performance liquid chromatography, and sequenced by Edman N-terminal degradation.[31] Another good example is the tomato−*Fusarium oxysporum* pathosystem, where the first effector of root invading fungi (Six1) was identified following a strategy similar to the previously mentioned but using, in this case, mass spectrometry analysis.[32] After this work, other further protein effectors have been characterized in different fungi.[33]

The workflow of proteomic analysis includes the following major steps: experimental design, sampling, sample preparation, protein extraction, protein separation, mass spectrometry (MS) analysis, protein identification and quantification, and statistical analysis of the data obtained (Figure 1). In the case of fungi, the first steps of the proteomic workflow are essential. The choice of a good extraction protocol in a proteomic experiment is crucial because only proteins which have been properly extracted and

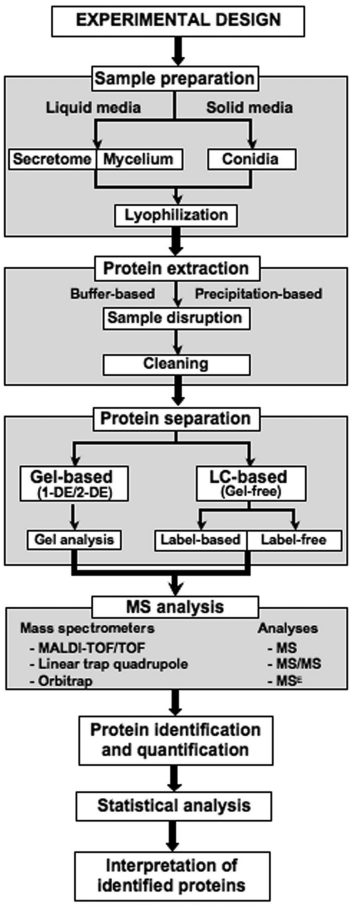


Figure 1. Schematic overview of proteomics workflow in plant pathogenic fungi.

solubilized may be detected and identified.[34] This is even more important in the case of recalcitrant organisms, such as plant pathogenic fungi. On one hand, they have a robust cell wall[35] that makes cell breakdown difficult and constitutes the majority of the cell mass. On the other hand, fungi produce polysaccharides, low-molecular organic acids, phenolic compounds, and other metabolites that may affect to the quality of protein preparation, as these molecules affect and interfere with the electrophoretic processes.[36] Several publications on plant pathogenic fungus proteomics are focused on the optimization of protein extraction protocols for *Rhizoctonia solani*,[37] and for extracellular proteins in higher basidiomycetes inhabiting lignocellulose[36] and in *Leptosphaeria maculans*.[38] Recently, a review has been published about proteomic workflow that discusses the problems derived from the protein extraction and separation prior to work with fungal samples.[2]

Important information on biological systems and processes can be obtained by comparative proteomics studies. Much effort has been directed to catalogue mycelial, conidial, sclerotial, and secreted proteins across a range of fungal species by establishing reference proteome maps of these fungal structures.[2] Several papers reporting changes in the protein profile between species, races, mutants, growth and developmental stages, as well as in growth conditions, have been published and reviewed.[2] Plant pathogenic fungus pathogenicity requires the coordinated regulation of multiple genes (and their protein products) which are involved in host recognition, spore germination, hyphal penetration, appressorium formation, toxin production, and secretion.[18,39] To study the infection cycle and to identify virulence factors, proteomics may be a successful tool for analyzing changes in protein expression between races and stages. However, most of these studies are made *in planta* after the plant inoculation. The major limitations in infected plant tissues are due to the fact that the fungal biomass and, as consequence, the pathogen proteins are a small portion of the total sample, so gels cannot distinguish between the two elements.

An interesting topic of study is the production of conidia, as this structure is the main source of disease dissemination, being a critical process in the spore dispersal and virulence of the fungus. Nevertheless, proteomic studies using conidia are scarce. Sulc et al.[40] have reported the protein profile of intact *Aspergillus* ssp. spores (including some plant pathogenic species) by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) MS. They built up a mass spectral database with 24 *Aspergillus* strains. These mass fingerprinting generated by MS may be used for typing and characterizing different fungal strains, as well as for finding new biomarkers in host−pathogen interactions. Another study was made on *Blumeria graminis f. sp. hordei* (Bgh), a fungus that causes powdery mildew on grasses, including cereals.[41] The proteome from Bgh conidia was analized using a combination of bidimensional electrophoresis (2-DE) and MS analyses. A total of 23 distinct fungal gene products were identified by searching at the NCBInr ESTs database, most of them with a predicted function in carbohydrate, lipid, or protein metabolism. These facts showed that the conidiospore is geared for the breakdown of storage compounds and metabolites during germination, correlating with previously reported transcriptomic data.[42,43] This research allowed a functionally annotated proteome for Bgh conidia. Finally, the comparison of conidial proteomes of *COM1* (conidial morphology regulating gene encoding putative transcriptional regulator) deletion in *Magnaporthe oryzae* (the main cause responsible for the restrictions of world rice production) mutant and its isogenic wild-type strain were performed. The *COM1* deletion in *M. oryzae* derived in aberrant conidial shape, reduced conidiation, and attenuated virulence. A 2-DE combined with MALDI-TOF MS analysis was employed to identify the molecular components regulated by the Com1 protein (Com1p) that might contribute to the aberrant phenotypes in this fungus.[44] A total of 31 proteins were identified that exhibited statistically significant alterations in their abundance levels. The data obtained in this study suggest that the Com1p may play a key role in transcriptional reprogramming of genes implicated in melanin biosynthesis, carbon and energy metabolism, structural organization of cell, lipid metabolism, or amino acid metabolism. Data validation by semiquantitative RT-PCR analysis revealed the down-regulation of genes encoding enzymes involved in melanin biosynthesis in the Δ*com1* mutant. These results suggest that the Com1p may regulate the transcription of genes involved in several cellular processes necessary for conidial development and appressorial penetration.[44]

A defining characteristic of plant pathogenic fungi is the secretion of a large number of degradative enzymes and other proteins, which have diverse functions in nutrient acquisition, substrate colonization, and ecological interactions.[45−47] Several extracellular fungal enzymes (e.g., polygalacturonases, pectate lyases, xylanases, and lipases) have been shown or postulated to be required for virulence in at least one host−pathogen interaction.[48−55] Many of these proteins are of special interest in the study of plant pathogens because they may be potencial targets for new fungicides. The first complete proteomic study of

dx.doi.org/10.1021/pr200873p |*J. Proteome Res.* 2012, 11, 3−16

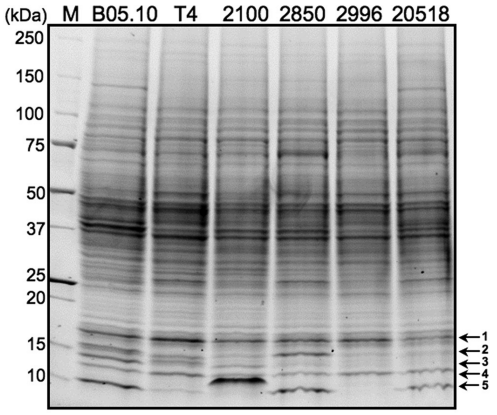


Figure 2. Protein profiles of six *B. cinerea* wild-type strains (B05.10, T4, 2100, 2850, 2996, 20518) that differ in host and virulence. This approach allows the assessment of differences in the protein band patterns among strains (indicated by arrows).

secreted proteins was released on the phytopathogenic fungus *Aspergillus flavus*.[56,57] The interest of this work was the ability of both *A. flavus* and *Aspergillus parasiticus* to degrade the flavonoids that plants produce as typical defense secondary metabolites against invading microorganisms. The secreted proteins were analyzed by 2-DE and MALDI-TOF MS, with 15 rutin-induced proteins and 7 noninduced proteins identified, including enzymes of rutine catabolism pathway and glycosidases.

Despite the existence of quite a number of different methods developed for protein extraction and separation,[2] they are not efficient enough to analyze entire proteomes. However, even the most simple monodimensional electrophoresis (1-DE) technique could be very valuable, especially in the case of comparative proteomics with large numbers of samples to be compared. One of the most important advantages of this technique is that it allows the detection of 2-DE elusive proteins such as hydrophobic, extreme p*I*, and low-molecular-weight ones.[58] For instance, 1-DE has made possible the distinction between phenotypes of different *B. cinerea* wild-type strains (with different host and virulence), providing relevant information about the intracellular protein profiles (Figure 2).[59] Most of the reported works use classical gel-based techniques (1- or 2-DE) coupled to MS or tandem MS (MS/MS), or shotgun proteomics based on liquid chromatography (LC)−MS/MS as proteomic strategy. Some methodologies have proven to be more powerful and decisive than others, according to the number of proteins identified. This is the case of Multidimensional Protein Identification Technology (MudPIT or LC/LC−MS/MS), a shotgun gel-free strategy that consists of a two-dimesional chromatography separation prior to MS. Moreover, it allows the identification of a much larger number of proteins compared to gel-based methods.[22,60,61] This technology permits the detection and identification of proteins rarely seen in 2-DE-based proteome analysis, including low-abundance proteins, such as transcription factors and protein kinases.[22] MudPIT was used to analyze the mechanisms of germling growth in *Uromyces appendiculatus* by comparing germinating asexual uredospores with inactive spores.[60] The proteins identified revealed that, after germination, there were few changes in the amount of accumulated proteins involved in glycolysis, acetyl Co-A metabolism, citric acid cycle, ATP-coupled proton transport, or gluconeogenesis. Moreover, the total amount of translation elongation factors remained high, supporting a prior model that suggests that germlings acquire protein translation machinery from uredospores. Nonetheless, germlings contained a higher amount of proteins involved in mitochondrial ADP:ATP translocation, which is indicative of increased energy production. There were more accumulating histone proteins, pointing to the reorganization of the nuclei that occurs after germination prior to appressorium formation. These changes showed that uredospores require high energy and structural proteins during germination, indicating a metabolic transition from dormancy to germination.[60]

Not only is the use of proteomics aimed to identify protein species (main objective of the descriptive proteomics), but also to quantify them; however, absolute—instead of relative—protein quantitation remains as one of the main challenges in MS-based quantitative proteomics.[62] There are different methods to dissect the proteome in a quantitative manner (for reviews see ref 63−65), both at the protein or peptide separation (gel or gel-free approaches), or MS stages (label or label-free approaches). Gel-based approaches use mainly 2-DE with poststaining, such as colloidal Comassie blue staining and fluorescence staining or prelabeling such as two-dimensional fluorescence difference gel electrophoresis (2-DIGE).[66−69] Quantification by 2-DE presents an overall sensitivity, a high resolving power, and the ability to discriminate protein isoforms and proteins with post-translational modifications. Two-DIGE increased the potential of 2-DE as different samples could be labeled with specific dyes and analyzed on the same gel. However, theses techniques are laborious, of limited reproducibility, and semiquantitative. Besides, 2-DE shows poor quantification performance for proteins with particular features, such as extreme sizes (large or small), large hydrophobicity, low abundance, or extreme isoelectric point.

Although 2-DE remains as a standard tool for proteomic research, current efforts are directed toward alternative gel-free shotgun strategies to quantify proteins. The coupling of microscale separations (microcapillary or μLC) with automated MS/MS has enhanced the development of this methodology. By LC−MS/MS, the complex mixtures of proteins are digested to peptides (normally using trypsin), which are separated according to their hydrophobicity by μLC, and the eluted peptides are introduced into the mass spectrometer.[70] Recent reviews examine techniques, software, and statistical analyses used in gel-free quantitative proteomics, and discuss about its strengths and limitations.[70−74] The gel-free strategies have been categorized as label-based and label-free approaches (Figure 3). In label-based approaches, protein or peptides can be labeled with stable isotopes or isobaric tags in different ways, both *in vivo* and *in vitro*, with a number of techniques and versions with acronymous termed ICAT, iTRAQ, SILAC, AQUA, ^{18}O, ^{15}N, SISCAPA, and QconCAT (Figure 3). Features and characteristics of each one has been extensively reviewed.[29,62,70,75] For all of these strategies, first two or more distinct protein samples are differentially labeled, one with an isotopically "light" tag and the other with an isotopically "heavy" tag. After labeling, the samples are combined and the resulting pooled peptides are separated by LC, both using in a single dimension or multidimensional strategies (LC/LC), depending on the complexity of the mixture. Peptides common to both samples, although differentially isotopically labeled, retain the same chemical properties and behave similarly during their purification and separation. As a result, such peptides are detected as peak pairs, which differ in the mass determined by the isotope label employed. The mass spectral peak heights, or the peak areas, are compared to calculate relative

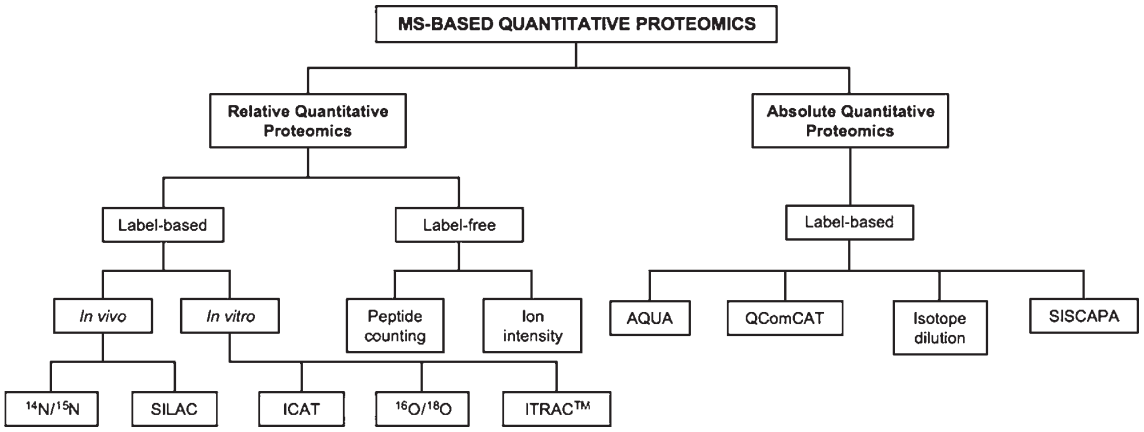


Figure 3. Scheme of quantitative proteomic methods. SILAC, stable isotope labeling in cell culture; ICAT, isotope-coded affinity tags; iTRAQ, isobaric tag for relative and absolute quantification; AQUA, absolute quantification; AComCAT, quantification used concatenated proteotypic peptides; SISCAPA, stable isotope standards and capture by antipeptide antibodies.

abundance levels for the peptides between the samples being compared. This allows the measurement of subtle changes in abundance between samples; proteins present only in one sample are detected as "singlet" peaks, with no corresponding partner peak detected.[70]

Label-free quantification compares two or more samples based on the measurements of ion intensity changes, such as peptide peak areas or peak heights in chromatography, and based on the spectral counting of identified proteins after MS/MS analysis (for reviews see refs 65, 73, 74). Peptide peak intensity or spectral counting is measured for individual LC−MS/MS or LC/LC−MS/MS runs, and changes in protein abundance are calculated via direct comparison between different analyses. The signal intensity method is based on the fact that this measurement is linearly proportional to the concentration of the measured peptide.[73] Spectral counting is based on the fact that more abundant peptides will be selected for fragmentation and will produce a higher abundance of MS/MS spectra. Therefore, the number of spectra is proportional to protein amount in data-dependent acquisition.[73] Ideally, samples for label-free comparisons are run consecutively on the same LC−MS/MS setup to avoid variations in ion intensities due to differences in the system setup (e.g., column properties, temperatures), and thereby allow precise reproduction of retention times.[74] Label-free quantification presents a lower accuracy (>30% uncertainty) compared to label-based methods. Nevertheless, label-free quantification has a larger dynamic range of protein concentration (2−3 logs) than label-based quantification (1−2 logs) and, in general, it is much simpler and cheaper.[29]

Shotgun proteomics is not an optimal technique for a systematic quantification of the identified proteins due to the stochastic nature and the limited sensitivity of the approach. Besides, only relative quantification of the abundant components can be usually performed.[62,76] An alternative MS approach, complementary to the shotgun proteomics, is the targeted proteomics that allows the quantification of predetermined sets of selected proteins. As a targeted MS experiment, the determination of specific MS coordinates is required, known as selected reaction monitoring (SRM) assays, used to measure specifically the proteins of interest.[62]

The potential of quantitative proteomics is far from being completely exploited in fungal plant pathogen research, and the use of these techniques should be a clear methodological objective in the future. For instance, Taylor et al. used iTRAQ to identify proteins and to quantify their relative abundance in *F. graminearum* when mycelia are producing mycotoxins, by mimicking plant infections with *in vitro* trichothecene-inducing conditions.[77] Cooper et al. semiquantified 461 proteins by MudPIT using spectral counting method at 95% or higher confidence level in *U. appendiculatus* germinating and inactive spores.[60] By the use of a gel-free/label-free method based on the measurements of ion intensity changes, mycelial and secreted proteins from two *B. cinerea* wild-type strains were identified (262 and 78 proteins, respectively), and about 50% of them were quantified (121 proteins in mycelium and 34 secreted proteins) with a high confidence level.[78,79] Other works used the combination of 1-DE and LC−MS/MS was used to analyze the changes in *B. cinerea* secretome under different growth conditions.[80−82] After 1-DE, bands were excised and digested using trypsin, and peptides were separated by LC and analyzed by MS/MS. The spectra generated in the MS/MS analysis were used to semi-quantify the proteins using the spectral counting method, and results will be discussed more extensively in the next section.

3. *B. cinerea*, *S. sclerotiorum*, AND *F. graminearum*: THE MOST STUDIED PLANT PATHOGENIC FUNGI IN PROTEOMICS

Among the large number of plant pathogenic fungi reported, only a few of them have been subjected to proteomic studies, because most of them are unsequenced "orphan" organisms, difficult to study as experimental system. However, in the last years, fungal proteomics has experienced great advances due to the increasing number of fungal genome sequencing projects. Moreover, the availability of EST database has increased the percentage of proteins identified.[41,83,84]

The substantial economic losses worldwide, both pre- and postharvest, caused by *B. cinerea*, *S. sclerotiorum*, and *F. graminearum*, have ensured that these three fungi are the most studied in plant pathogenic fungus proteomics at the moment due to their agronomic interest. Proteomic studies made on fungi with agricultural (as biocontrol agents) or biotechnological interest are outside the scope of this review, as well as those studies made on plant−fungus interactions. The results obtained in proteomic studies of these three fungi are commented below. Original papers published on plant

dx.doi.org/10.1021/pr200873p | *J. Proteome Res.* 2012, 11, 3–16

Table 2. Original Proteomics Papers Published on Plant Pathogenic Fungi with Interest for Agriculture

fungus	fungal material	experimental workflow	number of identified proteins	remarks	reference
Aspergillus ssp.	Conidia	1-DE, MALDI-TOF MS	11	Characterization and typing of intact fungal spores of 20 *Aspergillus* species by MS. Optimization of protein extraction.	40
Aspergillus flavus	Secretome	1-DE/2-DE, LC−MS/MS	22	Identification of secreted proteins from *A. flavus* grown on the flavonoid rutin (3-*O*-glycoside)-containing medium and on rich medium (potato dextrose broth).	57
	Secretome	1-DE/2-DE, MALDI-TOF-MS	51	Identification of secreted proteins from *A. flavus* grown on three different carbon sources (flavonoid rutin (3-*O*-glycoside), glucose and potato dextrose).	56
Blumeria graminis f. sp. *hordei*	Conidia	2-DE, MALDI-TOF/TOF-MS/MS	123	Description of conidium proteome.	41
	Haustorium	LC−MS/MS	204	Description of haustorium proteome.	83
Botrytis cinerea	Mycelium	2-DE, MALDI-TOF/TOF-MS/MS, LC−MS/MS	22	Description of mycelium proteome.	85
	Mycelium	2-DE, MALDI-TOF/TOF-MS/MS, LC-MS/MS	8	Comparative analysis of two strains differing in virulence and toxin production.	86
	Mycelium	2-DE, MALDI-TOF/TOF-MS/MS	306	Analysis of proteome during cellulose degradation.	87
	Secretome	1-DE, LC MS/MS	89	Analysis of secretome using minimal media supplemented with the extract of full red tomato, ripened strawberry or *Arabidopsis* leaf extract.	80
	Secretome	1-DE, LC MS/MS	131	Study of impact pectin esterification degree on secreted enzyme.	81
	Secretome	2-DE, MALDI-TOF/TOF-MS/MS	56	Analysis of secretome using minimal media supplemented with several carbon sources and plant-based elicitors.	88
	Secretome	2-DE, MALDI-TOF MS and 1-DE, LC−MS/MS	105	Analysis of early secretome using minimal media enriched with low molecular plant compounds, containing dialysis bag enclosing 50% tomato, strawberry or kiwifruit extracts.	82
Curvularia lunata	Mycelium	2-DE, MALDI-TOF/TOF-MS/MS	20	Analysis of the proteome profile of six different isolates in an attempt to correlate the band or spot pattern with virulence.	89

dx.doi.org/10.1021/pr200873p | *J. Proteome Res.* 2012, 11, 3–16

Table 2. Continued

fungus	fungal material	experimental workflow	number of identified proteins	remarks	reference
Fusarium graminearum	Secretome	1-DE/2-DE, LC-MS/MS	84	Analysis of the fungal secretome grown on glucose and on hop cell wall.	90
	Secretome	1-DE, LC−MS/MS	289	High-throughput analysis of proteins secreted by fungus grown *in vitro* (media supplemented with ten different carbon source) and *in planta* (wheat heads).	91
	Mycelium	2-DE, LC−MS/MS and iTRAQ	435 (130)[a]	Time course study of profile protein expression differences by inducing to produce trichothecenes *in vitro*.	77
	Mycelium	2-DE, LC−MS/MS	23	Study of effects produced by the virus (FgV-DK21) in the fungal development and virulence.	92
Leptosphaeria maculans	Secretome	1-DE, liquid-phase IEF, 2-DE		Proteomic protocols for extraction, concentration and resolution of fungal secretomes.	38
Magnaporthe grisea	Conidia	2-DE, MALDI-TOF MS	31	Study of effects produced by deletion of a conidial morphology regulating gene encoding putative transcriptional regulator (COM1) in the conidial shape, conidiation and virulence.	44
Phakopsora pachyrhyzi	Urediniospore	2-DE, MALDI-TOF/TOF MS/MS	117	Study of early events in the infection process.	93
Phytophthora infestans	Mycelium, sporangium, zoospore, cyst and germinated cyst	2-DE, MALDI-TOF-MS	3	Study of asexual life cycle.	94
	Cyst, germinated cyst and appressorium	2-DE, LC−MS/MS	13	Study of three developmental stages.	95
Phytophthora palmivora	Mycelium, sporangium, zoospore, cyst and germinated cyst	2-DE, MALDI-TOF-MS	3	Study of asexual life cycle.	94
Phytophthora ramorum	Cell wall	LC−MS/MS	17	Study of secreted proteins and cell wall-associated protein during plant infection.	96
Pyrenophora tritici-repentis	Mycelium and secretome	2-DE, LC−MS/MS	63	Evaluation of proteomes of both avirulent and virulent isolates of the fungus.	97
Pleorotus ostreatus	Secretome	1-DE, ^{18}O labeling, MALDI-TOF-MS, *de novo* sequencing-MS/MS	5	Identification of the protein responsible for 17β-hydroxysteroid dehydrogenase activity.	98
Rhizoctonia solani	Mycelium	2-DE, MALDI-TOF-MS	11	Optimization of protein extraction protocol for 2-DE.	37
Sclerotinia sclerotiorum	Mycelium and secretome	2-DE, LC−MS/MS	113	High-throughput identification.	99
	Sclerotium	2-DE, LC−MS/MS	64	Study of three stages of sclerotial development.	100
	Sclerotium exudates	2-DE, LC−MS/MS	56	Proteomic study of secreted proteins.	101

dx.doi.org/10.1021/pr200873p | J. Proteome Res. 2012, 11, 3–16

Table 2. Continued

fungus	fungal material	experimental workflow	number of identified proteins	remarks	reference
Serpula lacrymans	Secretome	LC−MS/MS	39	Comparative and functional genomic (transcriptome and proteome) analyses to study wood decomposition machinery, comparing several Agaricomycetes (species producing brown rot and white rot wood decay, parasitism, and mutualistic ectomycorrhyzal symbiosis).	28
Stagnospora nodorum	Mycelium	2-DE, LC−MS/MS	7	Identification of targets of the signaling pathway by analyzing a Galpha-defective mutant proteome.	102
	Mycelium	iTRAQ, 2-D LC−MALDI-MS/MS	590 (49)[a]	Characterization of protein expression changes in Galpha-defective mutant.	103
Thielaviopsis basicola	Mycelium	2-DE, LC−MS/MS	41	Study of proteome an *in vitro* growing fungus in presence of root extracts from nonhost (wheat, hairy vetch) and susceptible host (cotton, lupin) plants.	104
	Mycelium	2-DE, LC−MS/MS	43	Genomic, proteomic and virulence studies of several isolates.	105
Uromyces appendiculatus	Uredospores	MudPIT	461	Study of proteome of germinating asexual uredospores.	60
Ustilago maydis	Mycelium	2-DE, MALDI-TOF-MS, LC−MS/MS	250	Study of mycelium proteome.	106
Verticillium dahliae	Mycelium	2-DE, LC−MS/MS	18	Comparison of proteomes of highly (Vd1396-9) and weakly (Vs06-14) aggressive isolates.	107

[a] Quantified proteins are indicated between parentheses.

dx.doi.org/10.1021/pr200873p | J. Proteome Res. 2012, 11, 3–16

pathogenic fungi interesting for agriculture using proteomics are listed in Table 2.

3.1. *B. cinerea*

B. cinerea Pers. Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) is an important necrotrophic pathogen of nursery plants, vegetables, ornamental, field and orchard crops, as well as of stored and transported agricultural products, which causes significant economic losses in agriculture.[108] In the last years, this fungus has become a model organism to study plant pathogenic fungi. The first proteomic study using this species was published in 2006, and described a partial proteome from mycelium protein extracts.[85] Four hundred spots were detected in Coomassie-stained 2-DE gels, covering the 5−8 pH and 14−85 kDa ranges. From a total of 60 spots subjected to MS analysis, 22 proteins were identified by MALDI-TOF MS or electrospray ion trap MS (ESI IT MS/MS). Some of these proteins corresponded to forms of malate dehydrogenase (MDH), glyceraldehyde-3-phosphate dehydrogenase (GADPH), and a kind of cyclophilin which have been related to virulence.[85] In a second study, comparative proteomic analysis of mycelium of two *B. cinerea* strains, differing in virulence and toxin production, revealed the existence of 73 qualitative and quantitative differences in the 2-DE protein profile. A total of 28 spots were identified by MALDI-TOF MS and ESI IT MS/MS. The same proteins mentioned above were identified, and appeared over-expressed or only in the most virulent strain.[86] Seventeen spots were identified as MDH and up-accumulated in the most virulent strain. This enzyme catalyzes the reversible conversion of oxalacetate and malate. Oxalacetate is an oxalic acid precursor that has been described as a pathogenicity factor in *B. cinerea*.[86] The secretion of oxalic acid creates an acidic environment that may generate a more suitable, ecological niche for the pathogenic activities of the fungus. In addition, environmental acidification generated by oxalic acid would lead to the biosynthesis and secretion of phytotoxins as botrydial and dihydrobotrydial. Four spots were identified as GADPH. Apart from its well-known role in the glycolytic cycle, GAPDH has been reported to influence many other cellular processes and to act as a virulence factor in different organisms.[86] The four spots identified as GAPDH in this study were only present in the more virulent strain, supporting the hypothesis that the oxidative metabolism in this strain may be more active than that of the less virulent strain, and suggesting a putative role as virulence factor in *B. cinerea*.[86] A cytosolic cyclophilin, present in the most pathogenic strain, was absent in the proteome of the less pathogenic strain. These proteins have been shown to be involved in many different cellular processes, including their role as virulence determinants in fungal pathogens of human and plant cells.[86] The role of cyclophilin as virulence factor in *B. cinerea* has been previously shown and proposed to be involved in the later stages of infection, such as penetration or plant colonization.[86] A third and more exhaustive work tried to establish a mycelium proteomic map of *B. cinerea* during cellulose degradation.[87] Using 2-DE combined with MALDI-TOF/TOF MS/MS, 306 proteins were identified. The authors concluded that since cellulose is one of the major components of the plant cell wall, many of the identified proteins may have a crucial role in the pathogenicity process, may be involved in the infection cycle, and may be potential antifungal targets. Because of the lack of fungal sequenced genomes at that moment, the number of proteins identified in these studies was limited, and mostly representing unannotated or hypothetical proteins.[87]

The secretome of phytopathogenic fungi is presumed to be a key element of their infection strategy. Four studies of the proteins secreted by *B. cinerea* have been published to dissect the complexity and versatility of the *B. cinerea* secretome.[80−82,88] These studies identified 89, 131, 56, and 105 proteins, respectively. Moreover, different fungal strains, growth conditions, and proteomic approaches were applied. The availability of these data sets gives us the opportunity to compare the *B. cinerea* secretome in quite different conditions. In the first work, the secretome produced by this fungus in response to a mock interaction using extracts of full red tomato, ripened strawberry, and *Arabidopsis* leaves, and employing growth on a solid surface, was analyzed.[80] Overall, 89 *B. cinerea* proteins were identified by shotgun high-throughput LC−MS/MS from all growth conditions. Sixty of these proteins were predicted to contain a signal peptide sequence indicating the extracellular location of the proteins. The identified proteins were involved in carbohydrate metabolism, oxidation/reduction reactions, or were transport proteins, peptidases, or pathogenicity factors. *B. cinerea* was shown to secrete a wide array of enzymes facilitating host tissue penetration and colonization to infect a variety of hosts. There were significant changes in the relative abundance and composition of the secreted enzymes depending on the media composition. In the presence of more favorable food sources, such as full ripe strawberry and tomato extracts, the fungus increased the protein secretion when compared to *Arabidopsis* leaf extract. These data could provide important insights into how *B. cinerea* might use secreted proteins for plant infection and colonization.[80] In the second work, the impact of pectin esterification degree on secreted enzyme of *B. cinerea* was studied, because changes during the ripening process of fruits seem to play an important role in the activation of the dormant infection. By the combination of 1-DE and LC−MS/MS, 126 proteins were identified and 87 proteins were predicted as secreted by SignalP (http://www.cbs.dtu.dk/services/SignalP/), some of them being pectinases.[81] The results showed that the growth of *B. cinerea* and the secretion of proteins were similar in cultures containing differently esterified pectins. Therefore, it is likely that the activation of this fungus from the dormant state is not solely dependent on changes in the pectin esterification degree component of the plant cell wall.[81] In the third work, the secretion of discrete sets of proteins was induced by the controlled introduction of several carbon sources and plant-based elicitors (glucose, starch, pectin, carboxymethylcellulose (CMC), and tomato cell wall (TCW)).[88] The use of different carbon sources and plant-based elicitors resulted in different degrees of complexity of fungal response, moving from a state of constitutive fungal growth and a simple secretome (by using glucose as a nutrient) toward a more complex and possibly pathogenic secretory behavior (induced by TCW). The corresponding 2-DE profiles showed an increasing number and variety of spots when CMC or TCW were used. The secretion of proteins, most of which are cell wall-degrading enzymes, was similar in glucose, starch, and pectin treatments. Nevertheless, more proteins were detected after CMC treatment, and even more when TCW was the sole carbon source. A total of 78 spots were identified by MALDI-TOF/TOF MS/MS analysis, corresponding to 56 unique proteins and their respective isoforms, which represent 2.5% of the predicted secreted proteins from *B. cinerea*.[45] Sequences of all identified proteins contained secretion signal peptides for both classical or non-classical secretory pathways. Some of the identified proteins are involved in the pathogenicity process (e.g., pectin methylesterases, xylanases, proteases).[18,49] Proteins secreted at the beginning of the infection, during the germination of conidia

on the plant surfaces or wound are of special interest, as they may play essential roles in the establishment of a successful infection. In the fourth work, conidia from *B. cinerea* were germinated in conditions that resembled the plant environment (e.g., synthetic medium enriched with low molecular weight plant compounds, containing dialysis bag which enclosed 50% tomato, strawberry, or kiwifruit extracts), and the proteins secreted during the first 16 h were collected.[82] The 2-DE map of the precipitated secretome showed a similar spot pattern for all the conditions studied and for the control medium without plant extract (with glucose as carbon source). Of the 86 spots excised, only 16 were identified by MALDI-TOF MS corresponding to 11 unique proteins. Because of the low number of spots identified after 2-DE, protein extracts were subjected to 1-DE coupled with LC−MS/MS and consequently 105 proteins were identified. An interesting conclusion from these data is that the composition of the early secretome is not as variable as could be expected, so that *B. cinerea* seems to secrete during its germination a common set of proteins in every condition, which is completed by a lower number of proteins specific for certain conditions. Remarkable the high amount of proteases that were found in the secretome of *B. cinerea*.[80,82] These enzymes could be considered as potential virulence factors for fungi because, apart from their obvious role in generating amino acids to sustain fungal growth, proteases may also have important roles in two other aspects of fungal virulence: they could degradate (i) plant cell wall proteins to promote fungal hyphal penetration, and (ii) plant defense proteins to increase the chances for a successful infection. One of the most abundant proteins secreted and detected in all conditions was the aspartic protease BcAP8 or rhizopuspepsin-2 (BC1G_03070.1). The proteomic analysis of the Δ*bc1g_03070* mutant showed some differences in the pattern of bands/spots observed in the 1-DE or 2-DE gels. The absence of this protease in the secretome caused the decrease of up to 90% in the secreted protease activity. Furthermore, an increase in the intensity of the high molecular weight protein bands in the 1-DE profile and a decrease, or even the disappearance, of the smallest bands were caused. These results may suggest that the abundant secreted proteases of *B. cinerea* are causing significant cleavage of the secreted proteins and, as a consequence, render smaller fragments that, having smaller sequence coverage, are more difficult to identify.[82] Summarizing all the papers mentioned before, a total of 258 secreted protein species have been identified using different proteomic approaches (1-DE combined with LC−MS/MS and 2-DE combined with MALDI-TOF/TOF MS/MS), which represent a 10% of the predicted secreted proteins from *B. cinerea*,[45] in different growing conditions. The comparison of these data sets seems to imply that the *B. cinerea* secretome is highly adaptative, in the sense that very different sets of proteins are secreted when the growth conditions or the age of the mycelium differ.

Finally, the use of different proteomic approaches may help to validate the obtained data and to deepen in the characterization and comparison of proteomes. For instance, in the case of *B. cinerea*, a proteomic analysis including gel-based (1-DE and 2-DE) and gel-free (label-free) approaches has been carried out to characterize and validate a collection of *B. cinerea* mutants whose infection cycle is affected.[109]

3.2. *S. sclerotiorum*

A close relative to *B. cinerea* is the soil-borne *S. sclerotiorum*, which is a necrotrophic plant pathogen capable of infecting more than 400 plant species worldwide.[110] Yajima and Kav[99] performed the first comprehensive proteome-level study in this important phytopathogenic fungus, in order to gain a better understanding of its life cycle and ability to infect susceptible plants. A total of 18 secreted and 95 mycelial proteins were identified, by high-throughput identification combining 2-DE and LC−MS/MS. Many of the annotated secreted proteins were cell wall-degrading enzymes that had been previously identified as pathogenicity or virulence factors of *S. sclerotiorum*. However, one of them, α-L-arabinofuranosidase, which is involved in the initiation or progression of plant diseases, was not detected by previous EST studies, clearly demonstrating the merit of performing proteomic research. Furthermore, this study allowed the annotation of a number of proteins that were unnamed, predicted, or hypothetical proteins with undetermined functions in the available databases.

This fungus produces sclerotia which are long-term survival and dissemination structures that serve as the primary source of inoculum during seasonal crop infection cycles. Two recent proteomic analyses have been carried out in order to detect the proteins involved in the molecular events associated to sclerotial development. Liang et al.[100] reported the first proteomics-based analysis of sclerotial development. A total of 88 protein spots were observed by 2-DE to exhibit significant temporal differences in abundance at three representative stages of sclerotial development, and were identified using LC−MS/MS. Most of these proteins are involved in metabolism, energy, transcription and protein fate, cell defense, differentiation, and others with unknown functions. In addition, proteins involved in the process of melanogenesis were found to be differentially abundant during sclerotial development, such as the development-specific protein, Ssp. This protein may have a role in the formation and preservation of dormancy, and in the germination of sclerotia.[100] During sclerotial development, the exudation of liquid droplets is a common feature but little is known regarding the nature of these exudates. Moreover, the same authors performed a proteome-level study to identify the proteins present in the exudates.[101] A total of 56 proteins were identified, found to be and involved in metabolic pathways and biological energy production, as well as signal transduction, and those with unknown functions.

3.3. *F. graminearum*

The fungal pathogen *F. graminearum* (teleomorph *Gibberella zeae*) is the causal agent of Fusarium head blight in wheat, barley, and other grains, and Gibberella ear rot in maize in temperate climates worldwide.[111] It synthesizes trichothecene mycotoxins during plant host attack to facilitate the spread within the host, which can cause significant disease in humans.[111] To study proteins and pathways that are important for successful host invasion, Taylor et al.[77] conducted experiments where *F. graminearum* cells were grown in aseptic liquid culture conditions conducive to trichothecene and butenolide production in the absence of host plant tissue. This approach was based on mycotoxin synthesis associated with early stage plant infection. Samples were collected from a time course study and proteins were subjected to 2-DE, LC−MS/MS and iTRAQ analysis. Statistical analysis of a filtered data set of 435 proteins revealed 130 *F. graminearum* proteins that showed significant changes in expression, 72 of which were up-accumulated relative to their level at the initial phase of the time course. There was a good agreement between up-accumulated proteins identified by 2-DE-MS/MS and iTRAQ. RT-PCR and Northern hybridization confirmed that genes encoding proteins that were up-regulated based on iTRAQ were also transcriptionally active

under mycotoxin-producing conditions. Numerous candidate pathogenicity proteins were identified using this technique, including many predicted secreted proteins. Curiously, enzymes catalyzing reactions in the mevalonate pathway leading to trichothecene precursors were either not identified or only identified in one replicate, indicating that proteomics approaches cannot always prove biological characteristics. In other study, 2-DE with MS was used to compare the proteome of virus-free and virus (FgV-DK21) infected *F. graminearum* cultures.[92] The virus perturbs fungal developmental processes (such as sporulation, morphology, pigmentation), and attenuates its virulence. A total of 148 spots showing differences in abundance were identified. Among these, 33 spots were subjected to LC−MS/MS, and 23 were identified. Seven proteins (including sporulation-specific gene SPS2, triose phosphate isomerase, nucleoside diphosphate kinase, and woronin body major protein precursor) were up-accumulated, while 16 (including enolase, saccharopine dehydrogenase, flavohemoglobin, mannitol dehydrogenase, and malate dehydrogenase) were down-accumulated. Variations in protein abundance were investigated at the mRNA level by real-time RT-PCR analysis, which confirmed the proteomic data for 9 out of the representative 11 selected proteins.[92]

Phalip et al. studied the secretome of this fungus grown on glucose and on plant cell wall (*Humulus lupulus*, L.).[90] The culture medium was found to contain a larger amount of proteins, and these more diverse when the fungus grew on the cell wall. Using both 1-DE and 2-DE coupled with LC−MS/MS analysis and protein identification based on similarity searches, 84 unique proteins were identified in the cell wall-grown fungal secretome, and 45% were implicated in plant cell wall degradation. These cell wall-degradating enzymes were predominantly matches to putative carbohydrate active enzymes implicated in cellulose, hemicellulose, and pectin catabolism. As expected, *F. graminearum* grown on glucose produced relatively few cell wall-degrading enzymes. These results indicated that fungal metabolism is focused on the synthesis and secretion of a whole arsenal of enzymes able to digest almost the entire plant cell wall.[90] High-throughput LC−MS/MS was used to identify proteins secreted by *F. graminearum* growth *in vitro* (in media with ten different carbon sources) and *in planta* (during infection of wheat heads).[91] A total of 289 proteins (229 *in vitro* and 120 *in planta*) were identified with high statistical confidence. Forty-nine of the *in planta* proteins were not found in any of the *in vitro* conditions. The majority (91−100%) of the *in vitro* proteins had signal peptide sequences, in contraxt with only 56% of the *in planta* proteins. At least 13 of the nonsecreted proteins found only *in planta* were single-copy housekeeping enzymes, including enolase, triose phosphate isomerase, phosphoglucomutase, calmodulin, aconitase, and malate dehydrogenase. The presence of these proteins in the *in planta* but not *in vitro* secretome might indicate that significant fungal lysis occurs during pathogenesis. On the other hand, several of the proteins lacking signal peptides that were found *in planta* have been reported to be potent immunogens secreted by plant pathogenic fungi, and therefore could be important in the interaction between *F. graminearum* and its host plants.[91]

4. FUTURE PERSPECTIVES

Proteomic tools are increasing their importance in the quest for virulence factors in plant pathogenic fungi. New developments in experimental methods, software, and algorithms have contributed to make this technique an effective tool for the description of plant pathogenic fungus proteomes. Proteomics is therefore a good strategy to study the interaction between plants and microbes mediated by excreted molecules, the role of the cell wall and the interface. It may also be used to identify fungal protein effectors facilitating either infection (e.g., virulence factors, enzymes of the toxin biosynthesis pathways) or trigger defense responses (e.g., avirulence factors), and thus to establish new targets which promote new strategies for environmentally friendly control of plant diseases. Nevertheless, the complete characterization of the proteome remains as a huge challenge, and the dynamic range needed to obtain a complete proteome has not been achieved yet. We must consider proteomics as a part of a multidisciplinary approach, including a combination of high-throughtput -omics (genomics, transcriptomics, proteomics, and metabolomics). Therefore, another challenge for the future will be to integrate the vast amount of data that is being generated and to store it in databases and repositories for further use by the scientific community.

Over the past decade, gel-free proteomics techniques (LC−MS/MS or LC−LC−MS/MS) have emerged as complementary methods to the existing gel-based 2-DE, because the limitations of 2-DE and MS analyses showed little information. The use of the second- and third-generation techniques for quantitative proteomics is another challenge for the future in plant pathogenic fungi proteomics. Thus, gel-free techniques (e.g., SRM) should be used for quantitative proteomics; although the availability of and cost of operation associated with high-throughtput LC−MS instrumentation (e.g., orbitrap) is restricted to relatively few laboratories and needs expert professionals to operate. Therefore, the overall process would require a reduction in cost and complexity for these more sensitive techniques to become routine analyses in the study of plant pathogenic fungi biology.

AUTHOR INFORMATION

Corresponding Author

*E-mail: q42gofer@uco.es. Phone: +34 957 218 574. Fax: +34 957 219 439.

ACKNOWLEDGMENT

This work was supported by the Spanish Ministry of Science and Innovation (BotBank Project, EUI2008-03686), the Andalucian Government "Junta de Andalucía", and the University of Cordoba (AGR-0164: Agroforestry and Plant Biochemistry and Proteomics Research Group).

REFERENCES

(1) Hawksworth, D. L.; Kirk, P. M.; Sutton, B. C.; Pegler, D. N. *Ainsworth and Bisby's Dictionary of the Fungi*, 9th ed.; Kirk, P. M., Cannon, P. F., David, J. C., Stalpers, J. A., Eds.; International Mycological Institute, Surrey, England: Wallingford, Oxon, U.K., 2001.

(2) Gonzalez-Fernandez, R.; Prats, P.; Jorrin-Novo, J. V. Proteomics of plant pathogenic fungi. *J. Biomed. Biotechnol.* **2010**, *2010*, No. Article ID 932527. DOI: 10.1155/2010/932527.

(3) Kikot, G. E.; Hours, R. A.; Alconada, T. M. Contribution of cell wall degrading enzymes to pathogenesis of Fusarium graminearum: A review. *J. Basic Microbiol.* **2009**, *49* (3), 231–241.

(4) van Esse, H. P.; Van't Klooster, J. W.; Bolton, M. D.; Yadeta, K. A.; van Baarlen, P.; Boeren, S.; Vervoort, J.; de Wit, P. J.; Thomma, B. P. The Cladosporium fulvum virulence protein Avr2 inhibits host proteases required for basal defense. *Plant Cell* **2008**, *20* (7), 1948–1963.

(5) Friesen, T. L.; Faris, J. D.; Solomon, P. S.; Oliver, R. P. Host-specific toxins: effectors of necrotrophic pathogenicity. *Cell. Microbiol.* **2008**, *10* (7), 1421–1428.

(6) Gao, X.; Kolomiets, M. V. Host-derived lipids and oxylipins are crucial signals in modulating mycotoxin production by fungi. *Toxin Rev.* **2009**, *28* (2−3), 79–88.

(7) Kim, K. S.; Min, J. Y.; Dickman, M. B. Oxalic acid is an elicitor of plant programmed cell death during Sclerotinia sclerotiorum disease development. *Mol. Plant−Microbe Interact.* **2008**, *21* (5), 605–612.

(8) Lawrence, C. B.; Mitchell, T. K.; Craven, K. D.; Cho, Y.; Cramer, R. A.; Kim, K. H. At death's door: Alternaria pathogenicity mechanisms. *Plant Phatol. J.* **2008**, *24* (2), 101–111.

(9) McCouch, S. Diversifying selection in plant breeding. *PLoS Biol.* **2004**, *2* (10), e347.

(10) Yang, Y.; Zhang, H.; Li, G.; Li, W.; Wang, X.; Song, F. Ectopic expression of MgSM1, a Cerato-platanin family protein from Magnaporthe grisea, confers broad-spectrum disease resistance in Arabidopsis. *Plant Biotechnol. J.* **2009**, *7* (8), 763–777.

(11) Kim, B. S.; Hwang, B. K. Microbial fungicides in the control of plant diseases. *J. Phytopathol.* **2007**, *155* (11−12), 641–653.

(12) Berg, G. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.* **2009**, *84* (1), 11–18.

(13) Arora, D. K.; Bridge, P. D.; Bhatnagar, D. *Fungal Biotechnology in Agricultural, Food and Environmental Applications*; Marcel Dekker: New York, 2004.

(14) Van De Wouw, A. P.; Howlett, B. J. Fungal pathogenicity genes in the age of 'omics'. *Mol. Plant Pathol.* **2011**, *12* (5), 507–514.

(15) Winnenburg, R.; Urban, M.; Beacham, A.; Baldwin, T. K.; Holland, S.; Lindeberg, M.; Hansen, H.; Rawlings, C.; Hammond-Kosack, K. E.; Kohler, J. PHI-base update: Additions to the pathogen host interaction database. *Nucleic Acids Res.* **2008**, *36* (Database issue), D572–576.

(16) Afroz, A.; Ali, G. M.; Mir, A.; Komatsu, S. Application of proteomics to investigate stress-induced proteins for improvement in crop protection. *Plant Cell Rep.* **2011**, *30* (5), 745–763.

(17) Egan, M. J.; Talbot, N. J. Genomes, free radicals and plant cell invasion: recent developments in plant pathogenic fungi. *Curr. Opin. Plant Biol.* **2008**, *11* (4), 367–372.

(18) Choquer, M.; Fournier, E.; Kunz, C.; Levis, C.; Pradier, J.-M.; Simon, A.; Viaud, M. Botrytis cinerea virulence factors: new insights into a necrotrophic and polyphageous pathogen. *FEMS Microbiol. Lett.* **2007**, *277* (1), 1–10.

(19) Hadwiger, L. A. Localization predictions for gene products involved in non-host resistance responses in a model plant/fungal pathogen interaction. *Plant Sci.* **2009**, *177* (4), 257–265.

(20) Tan, K. C.; Ipcho, S. V.; Trengove, R. D.; Oliver, R. P.; Solomon, P. S. Assessing the impact of transcriptomics, proteomics and metabolomics on fungal phytopathology. *Mol. Plant Pathol.* **2009**, *10* (5), 703–715.

(21) Walter, S.; Nicholson, P.; Doohan, F. M. Action and reaction of host and pathogen during *Fusarium* head blight disease. *New Phytol.* **2010**, *185* (1), 54–66.

(22) Washburn, M. P.; Wolters, D.; Yates, J. R., III. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat. Biotechnol.* **2001**, *19* (3), 242–247.

(23) Wolters, D. A.; Washburn, M. P.; Yates, J. R., III. An automated multidimensional protein identification technology for shotgun proteomics. *Anal. Chem.* **2001**, *73* (23), 5683–5690.

(24) Doyle, S. Fungal proteomics: From identification to function. *FEMS Microbiol. Lett.* **2011**, *321* (1), 1–9.

(25) Bhadauria, V.; Banniza, S.; Wang, L.-X.; Wei, Y.-D.; Peng, Y.-L. Proteomic studies of phytopathogenic fungi, oomycetes and their interactions with hosts. *Eur. J. Plant Pathol.* **2010**, *126* (1), 81–95.

(26) Mathesius, U. Comparative proteomic studies of root-microbe interactions. *J. Proteomics* **2009**, *72* (3), 353–366.

(27) 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.; Rocha, T. L. Plant-pathogen interactions: What is proteomics telling us? *FEBS J.* **2008**, *275* (15), 3731–3746.

(28) Eastwood, D. C.; Floudas, D.; Binder, M.; Majcherczyk, A.; Schneider, P.; Aerts, A.; Asiegbu, F. O.; Baker, S. E.; Barry, K.; Bendiksby, M.; Blumentritt, M.; Coutinho, P. M.; Cullen, D.; de Vries, R. P.; Gathman, A.; Goodell, B.; Henrissat, B.; Ihrmark, K.; Kauserud, H.; Kohler, A.; LaButti, K.; Lapidus, A.; Lavin, J. L.; Lee, Y. H.; Lindquist, E.; Lilly, W.; Lucas, S.; Morin, E.; Murat, C.; Oguiza, J. A.; Park, J.; Pisabarro, A. G.; Riley, R.; Rosling, A.; Salamov, A.; Schmidt, O.; Schmutz, J.; Skrede, I.; Stenlid, J.; Wiebenga, A.; Xie, X.; Kues, U.; Hibbett, D. S.; Hoffmeister, D.; Hogberg, N.; Martin, F.; Grigoriev, I. V.; Watkinson, S. C. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. *Science* **2011**, *333* (6043), 762–765.

(29) de Oliveira, J. M.; de Graaff, L. H. Proteomics of industrial fungi: trends and insights for biotechnology. *Appl. Microbiol. Biotechnol.* **2011**, *89* (2), 225–237.

(30) Harman, G. E.; Howell, C. R.; Viterbo, A.; Chet, I.; Lorito, M. Trichoderma species—opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* **2004**, *2* (1), 43–56.

(31) Schottens-Toma, I. M. J.; de Wit, P. J. G. M. Purification and primary structure of a necrosis-inducing peptide from the apoplastic fluids of tomato infected with Cladosporium fulvum (syn. Fulvia fulva). *Physiol. Mol. Plant Pathol.* **1988**, *33* (1), 59–67.

(32) Rep, M.; van der Does, H. C.; Meijer, M.; van Wijk, R.; Houterman, P. M.; Dekker, H. L.; de Koster, C. G.; Cornelissen, B. J. A small, cysteine-rich protein secreted by Fusarium oxysporum during colonization of xylem vessels is required for I-3-mediated resistance in tomato. *Mol. Microbiol.* **2004**, *53* (5), 1373–1383.

(33) Rep, M. Small proteins of plant-pathogenic fungi secreted during host colonization. *FEMS Microbiol. Lett.* **2005**, *253* (1), 19–27.

(34) Jorrin-Novo, J. V.; Maldonado, A. M.; Echevarria-Zomeno, S.; Valledor, L.; Castillejo, M. A.; Curto, M.; Valero, J.; Sghaier, B.; Donoso, G.; Redondo, I. Plant proteomics update (2007−2008): Second-generation proteomic techniques, an appropriate experimental design, and data analysis to fulfill MIAPE standards, increase plant proteome coverage and expand biological knowledge. *J. Proteomics* **2009**, *72* (3), 285–314.

(35) Ruiz-Herrera, J. *Fungal Cell Wall: Structure, Synthesis and Assembly*; CRC Press: Boca Raton, FL, 1992.

(36) Fragner, D.; Zomorrodi, M.; Kues, U.; Majcherczyk, A. Optimized protocol for the 2-DE of extracellular proteins from higher basidiomycetes inhabiting lignocellulose. *Electrophoresis* **2009**, *30* (14), 2431–2441.

(37) Lakshman, D. K.; Natarajan, S. S.; Lakshman, S.; Garrett, W. M.; Dhar, A. K. Optimized protein extraction methods for proteomic analysis of Rhizoctonia solani. *Mycologia* **2008**, *100* (6), 867–875.

(38) Vincent, D.; Balesdent, M. H.; Gibon, J.; Claverol, S.; Lapaillerie, D.; Lomenech, A. M.; Blaise, F.; Rouxel, T.; Martin, F.; Bonneu, M.; Amselem, J.; Dominguez, V.; Howlett, B. J.; Wincker, P.; Joets, J.; Lebrun, M. H.; Plomion, C. Hunting down fungal secretomes using liquid-phase IEF prior to high resolution 2-DE. *Electrophoresis* **2009**, *30* (23), 4118–4136.

(39) van Kan, J. A. L. Licensed to kill: The lifestyle of a necrotrophic plant pathogen. *Trends Plant Sci.* **2006**, *11* (5), 247–253.

(40) Sulc, M.; Peslova, K.; Zabka, M.; Hajduch, M.; Havlicek, V. Biomarkers of Aspergillus spores: Strain typing and protein identification. *Int. J. Mass Spectrom.* **2009**, *280*, 162–168.

(41) Noir, S.; Colby, T.; Harzen, A.; Schmidt, J.; Panstruga, R. A proteomic analysis of powdery mildew (Blumeria graminis f.sp. hordei) conidiospores. *Mol. Plant Pathol.* **2009**, *10* (2), 223–236.

(42) Both, M.; Csukai, M.; Stumpf, M. P.; Spanu, P. D. Gene expression profiles of Blumeria graminis indicate dynamic changes to primary metabolism during development of an obligate biotrophic pathogen. *Plant Cell* **2005**, *17* (7), 2107–2122.

(43) Both, M.; Eckert, S. E.; Csukai, M.; Muller, E.; Dimopoulos, G.; Spanu, P. D. Transcript profiles of Blumeria graminis development during infection reveal a cluster of genes that are potential virulence determinants. *Mol. Plant−Microbe Interact.* **2005**, *18* (2), 125–133.

(44) Bhadauria, V.; Wang, L. X.; Peng, Y. L. Proteomic changes associated with deletion of the Magnaporthe oryzae conidial morphology-regulating gene COM1. *Biol. Direct* **2010**, *5*, 61.
(45) Choi, J.; Park, J.; Kim, D.; Jung, K.; Kang, S.; Lee, Y. H. Fungal secretome database: Integrated platform for annotation of fungal secretomes. *BMC Genomics* **2010**, *11*, 105.
(46) Deising, H. B.; Kamoun, S. The Secretome of Plant-Associated Fungi and Oomycetes. In *The Mycota*; Springer: Berlin Heidelberg, 2009; Vol. 5, pp 173−180.
(47) Walton, J. D. Deconstructing the cell wall. *Plant Physiol.* **1994**, *104* (4), 1113–1118.
(48) King, B. C.; Waxman, K. D.; Nenni, N. V.; Walker, L. P.; Bergstrom, G. C.; Gibson, D. M. Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. *Biotechnol. Biofuels* **2011**, *4*, 4.
(49) Brito, N.; Espino, J. J.; Gonzalez, C. The endo-beta-1,4-xylanase xyn11A is required for virulence in Botrytis cinerea. *Mol. Plant−Microbe Interact.* **2006**, *19* (1), 25–32.
(50) Voigt, C. A.; Schafer, W.; Salomon, S. A secreted lipase of Fusarium graminearum is a virulence factor required for infection of cereals. *Plant J.* **2005**, *42* (3), 364–375.
(51) Oeser, B.; Heidrich, P. M.; Muller, U.; Tudzynski, P.; Tenberge, K. B. Polygalacturonase is a pathogenicity factor in the Claviceps purpurea/rye interaction. *Fungal Genet. Biol.* **2002**, *36* (3), 176–186.
(52) Isshiki, A.; Akimitsu, K.; Yamamoto, M.; Yamamoto, H. Endopolygalacturonase is essential for citrus black rot caused by Alternaria citri but not brown spot caused by Alternaria alternata. *Mol. Plant−Microbe Interact.* **2001**, *14* (6), 749–757.
(53) Yakoby, N.; Beno-Moualem, D.; Keen, N. T.; Dinoor, A.; Pines, O.; Prusky, D. Colletotrichum gloeosporioides pelB is an important virulence factor in avocado fruit-fungus interaction. *Mol. Plant−Microbe Interact.* **2001**, *14* (8), 988–995.
(54) ten Have, A.; Mulder, W.; Visser, J.; van Kan, J. A. The endopolygalacturonase gene Bcpg1 is required for full virulence of Botrytis cinerea. *Mol. Plant−Microbe Interact.* **1998**, *11* (10), 1009–1016.
(55) Deising, H.; Nicholson, R. L.; Haug, M.; Howard, R. J.; Mendgen, K. Adhesion pad formation and the involvement of cutinase and esterases in the attachment of uredospores to the host cuticle. *Plant Cell* **1992**, *4* (9), 1101–1111.
(56) Medina, M. L.; Haynes, P. A.; Breci, L.; Francisco, W. A. Analysis of secreted proteins from Aspergillus flavus. *Proteomics* **2005**, *5* (12), 3153–3161.
(57) Medina, M. L.; Kiernan, U. A.; Francisco, W. A. Proteomic analysis of rutin-induced secreted proteins from Aspergillus flavus. *Fungal Genet. Biol.* **2004**, *41* (3), 327–335.
(58) Supek, F.; Peharec, P.; Krsnik-Rasol, M.; Smuc, T. Enhanced analytical power of SDS-PAGE using machine learning algorithms. *Proteomics* **2008**, *8* (1), 28–31.
(59) González-Fernández, R.; Redondo, I.; Jorrín-Novo, J. V. Proteomics in *Botrytis cinerea*. The Simplest 1-DE Reveals Differences in Protein Abundance among Strains; XV International Botrytis Symposium, Cádiz, Spain, 2010; p 82.
(60) Cooper, B.; Neelam, A.; Campbell, K. B.; Lee, J.; Liu, G.; Garrett, W. M.; Scheffler, B.; Tucker, M. L. Protein accumulation in the germinating Uromyces appendiculatus uredospore. *Mol. Plant−Microbe Interact.* **2007**, *20* (7), 857–866.
(61) Ye, M.; Jiang, X.; Feng, S.; Tian, R.; Zou, H. Advances in chromatographic techniques and methods in shotgun proteome analysis. *TrAC, Trends Anal. Chem.* **2007**, *26* (1), 80–84.
(62) Malmström, L.; Malmström, J.; Aebersold, R. Quantitative proteomics of microbes: Principles and applications to virulence. *Proteomics* **2011**, *11* (15), 2947–2956.
(63) Elliott, M. H.; Smith, D. S.; Parker, C. E.; Borchers, C. Current trends in quantitative proteomics. *J. Mass Spectrom.* **2009**, *44* (12), 1637–1660.
(64) Han, X.; Aslanian, A.; Yates, J. R., III. Mass spectrometry for proteomics. *Curr. Opin. Chem. Biol.* **2008**, *12* (5), 483–490.
(65) Zhu, W.; Smith, J. W.; Huang, C. M., Mass spectrometry-based label-free quantitative proteomics. *J. Biomed. Biotechnol.* **2010**, *2010*, No. Article ID 840518.
(66) Gorg, A.; Weiss, W.; Dunn, M. J. Current two-dimensional electrophoresis technology for proteomics. *Proteomics* **2004**, *4* (12), 3665–3685.
(67) Heinemeyer, J.; Scheibe, B.; Schmitz, U. K.; Braun, H. P. Blue native DIGE as a tool for comparative analyses of protein complexes. *J. Proteomics* **2009**, *72* (3), 539–544.
(68) Rabilloud, T.; Vaezzadeh, A. R.; Potier, N.; Lelong, C.; Leize-Wagner, E.; Chevallet, M. Power and limitations of electrophoretic separations in proteomics strategies. *Mass Spectrom. Rev.* **2009**, *28* (5), 816–843.
(69) Valledor, L.; Jorrin, J. Back to the basics: Maximizing the information obtained by quantitative two dimensional gel electrophoresis analyses by an appropriate experimental design and statistical analyses. *J. Proteomics* **2011**, *74* (1), 1–18.
(70) Roe, M. R.; Griffin, T. J. Gel-free mass spectrometry-based high throughput proteomics: tools for studying biological response of proteins and proteomes. *Proteomics* **2006**, *6* (17), 4678–4687.
(71) Becker, C. H.; Bern, M. Recent developments in quantitative proteomics. *Mutat. Res.* **2010**, *722* (2), 171–182.
(72) Mueller, L. N.; Brusniak, M. Y.; Mani, D. R.; Aebersold, R. An assessment of software solutions for the analysis of mass spectrometry based quantitative proteomics data. *J. Proteome Res.* **2008**, *7* (1), 51–61.
(73) Neilson, K. A.; Ali, N. A.; Muralidharan, S.; Mirzaei, M.; Mariani, M.; Assadourian, G.; Lee, A.; van Sluyter, S. C.; Haynes, P. A. Less label, more free: approaches in label-free quantitative mass spectrometry. *Proteomics* **2011**, *11* (4), 535–553.
(74) Schulze, W. X.; Usadel, B. Quantitation in mass-spectrometry-based proteomics. *Annu. Rev. Plant Biol.* **2010**, *61*, 491–516.
(75) Porteus, B.; Kocharunchitt, C.; Nilsson, R. E.; Ross, T.; Bowman, J. P. Utility of gel-free, label-free shotgun proteomics approaches to investigate microorganisms. *Appl. Microbiol. Biotechnol.* **2011**, *90* (2), 407–416.
(76) Gallien, S.; Duriez, E.; Domon, B. Selected reaction monitoring applied to proteomics. *J. Mass Spectrom.* **2011**, *46* (3), 298–312.
(77) Taylor, R. D.; Saparno, A.; Blackwell, B.; Anoop, V.; Gleddie, S.; Tinker, N. A.; Harris, L. J. Proteomic analyses of Fusarium graminearum grown under mycotoxin-inducing conditions. *Proteomics* **2008**, *8* (11), 2256–2265.
(78) González-Fernández, R.; Aloria, K.; Redondo, I.; Arizmendi, J. M.; Jorrin-Novo, J. V. Gel-free/label-free analysis to study the secretome of *Botrytis cinerea*. 10th Annual Congress - HUPO, Geneva, Switzerland, 2011.
(79) González-Fernández, R.; Aloria, K.; Redondo, I.; Arizmendi, J. M.; Jorrin-Novo, J. V. Gel-free/label-free analysis to study the proteome of *Botrytis cinerea*. 4th Congress of the Spanish Proteomics Society. New trends in Proteomics, Segovia, Spain, 2011; p 190.
(80) Shah, P.; Atwood, J. A.; Orlando, R.; El Mubarek, H.; Podila, G. K.; Davis, M. R. Comparative proteomic analysis of Botrytis cinerea secretome. *J. Proteome Res.* **2009**, *8* (3), 1123–1130.
(81) Shah, P.; Gutierrez-Sanchez, G.; Orlando, R.; Bergmann, C. A proteomic study of pectin-degrading enzymes secreted by Botrytis cinerea grown in liquid culture. *Proteomics* **2009**, *9* (11), 3126–3135.
(82) Espino, J. J.; Gutierrez-Sanchez, G.; Brito, N.; Shah, P.; Orlando, R.; Gonzalez, C. The Botrytis cinerea early secretome. *Proteomics* **2010**, *10* (16), 3020–3034.
(83) Godfrey, D.; Zhang, Z.; Saalbach, G.; Thordal-Christensen, H. A proteomics study of barley powdery mildew haustoria. *Proteomics* **2009**, *9* (12), 3222–3232.
(84) Skinner, W.; Keon, J.; Hargreaves, J. Gene information for fungal plant pathogens from expressed sequences. *Curr. Opin. Microbiol.* **2001**, *4* (4), 381–386.
(85) Fernandez-Acero, F. J.; Jorge, I.; Calvo, E.; Vallejo, I.; Carbu, M.; Camafeita, E.; Lopez, J. A.; Cantoral, J. M.; Jorrin, J. Two-dimensional electrophoresis protein profile of the phytopathogenic fungus Botrytis cinerea. *Proteomics* **2006**, *6* (Suppl. 1), S88–96.

(86) Fernandez-Acero, F. J.; Jorge, I.; Calvo, E.; Vallejo, I.; Carbu, M.; Camafeita, E.; Garrido, C.; Lopez, J. A.; Jorrin, J.; Cantoral, J. M. Proteomic analysis of phytopathogenic fungus Botrytis cinerea as a potential tool for identifying pathogenicity factors, therapeutic targets and for basic research. *Arch. Microbiol.* **2007**, *187* (3), 207–215.

(87) Fernandez-Acero, F. J.; Colby, T.; Harzen, A.; Cantoral, J. M.; Schmidt, J. Proteomic analysis of the phytopathogenic fungus Botrytis cinerea during cellulose degradation. *Proteomics* **2009**, *9* (10), 2892–2902.

(88) Fernandez-Acero, F. J.; Colby, T.; Harzen, A.; Carbu, M.; Wiencke, U.; Cantoral, J. M.; Schmidt, J. 2-DE proteomic approach to the Botrytis cinerea secretome induced with different carbon sources and plant-based elicitors. *Proteomics* **2010**, *10* (12), 2270–2280.

(89) Xu, S.; Chen, J.; Liu, L.; Wang, X.; Huang, X.; Zhai, Y. Proteomics associated with virulence differentiation of Curvularia lunata in maize in China. *J. Integr. Plant Biol.* **2007**, *49* (4), 487–496.

(90) Phalip, V.; Delalande, F.; Carapito, C.; Goubet, F.; Hatsch, D.; Leize-Wagner, E.; Dupree, P.; Dorsselaer, A. V.; Jeltsch, J. M. Diversity of the exoproteome of Fusarium graminearum grown on plant cell wall. *Curr. Genet.* **2005**, *48* (6), 366–379.

(91) Paper, J. M.; Scott-Craig, J. S.; Adhikari, N. D.; Cuomo, C. A.; Walton, J. D. Comparative proteomics of extracellular proteins in vitro and in planta from the pathogenic fungus Fusarium graminearum. *Proteomics* **2007**, *7* (17), 3171–3183.

(92) Kwon, S. J.; Cho, S. Y.; Lee, K. M.; Yu, J.; Son, M.; Kim, K. H. Proteomic analysis of fungal host factors differentially expressed by *Fusarium graminearum* infected with *Fusarium graminearum* virus-DK21. *Virus Res.* **2009**, *144* (1−2), 96–106.

(93) Luster, D. G.; McMahon, M. B.; Carter, M. L.; Fortis, L. L.; Nunez, A. Proteomic analysis of germinating urediniospores of Phakopsora pachyrhizi, causal agent of Asian soybean rust. *Proteomics* **2010**, *10* (19), 3549–3557.

(94) Shepherd, S. J.; van West, P.; Gow, N. A. Proteomic analysis of asexual development of Phytophthora palmivora. *Mycol. Res.* **2003**, *107* (Pt. 4), 395–400.

(95) Ebstrup, T.; Saalbach, G.; Egsgaard, H. A proteomics study of in vitro cyst germination and appressoria formation in Phytophthora infestans. *Proteomics* **2005**, *5* (11), 2839–2848.

(96) Meijer, H. J.; van de Vondervoort, P. J.; Yin, Q. Y.; de Koster, C. G.; Klis, F. M.; Govers, F.; de Groot, P. W. Identification of cell wall-associated proteins from Phytophthora ramorum. *Mol. Plant−Microbe Interact.* **2006**, *19* (12), 1348–1358.

(97) Cao, T.; Kim, Y. M.; Kav, N. N.; Strelkov, S. E. A proteomic evaluation of Pyrenophora tritici-repentis, causal agent of tan spot of wheat, reveals major differences between virulent and avirulent isolates. *Proteomics* **2009**, *9* (5), 1177–1196.

(98) Matis, M.; Zakelj-Mavric, M.; Peter-Katalinic, J. Mass spectrometry and database search in the analysis of proteins from the fungus Pleurotus ostreatus. *Proteomics* **2005**, *5* (1), 67–75.

(99) Yajima, W.; Kav, N. N. The proteome of the phytopathogenic fungus Sclerotinia sclerotiorum. *Proteomics* **2006**, *6* (22), 5995–6007.

(100) Liang, Y.; Rahman, M. H.; Strelkov, S. E.; Kav, N. N. Developmentally induced changes in the sclerotial proteome of Sclerotinia sclerotiorum. *Fungal Biol.* **2010**, *114* (8), 619–627.

(101) Liang, Y.; Strelkov, S. E.; Kav, N. N. The proteome of liquid Sclerotial exudates from Sclerotinia sclerotiorum. *J. Proteome Res.* **2010**, *9* (6), 3290–3298.

(102) Tan, K. C.; Heazlewood, J. L.; Millar, A. H.; Thomson, G.; Oliver, R. P.; Solomon, P. S. A signaling-regulated, short-chain dehydrogenase of Stagonospora nodorum regulates asexual development. *Eukaryotic Cell* **2008**, *7* (11), 1916–1929.

(103) Casey, T.; Solomon, P. S.; Bringans, S.; Tan, K. C.; Oliver, R. P.; Lipscombe, R. Quantitative proteomic analysis of G-protein signalling in Stagonospora nodorum using isobaric tags for relative and absolute quantification. *Proteomics* **2010**, *10* (1), 38–47.

(104) Coumans, J. V.; Moens, P. D.; Poljak, A.; Al-Jaaidi, S.; Pereg, L.; Raftery, M. J. Plant-extract-induced changes in the proteome of the soil-borne pathogenic fungus Thielaviopsis basicola. *Proteomics* **2010** *10* (8), 1573–1591.

(105) Coumans, J. V.; Harvey, J.; Backhouse, D.; Poljak, A.; Raftery, M. J.; Nehl, D.; Katz, M. E.; Pereg, L. Proteomic assessment of host-associated microevolution in the fungus Thielaviopsis basicola. *Environ. Microbiol.* **2011**, *13* (3), 576–588.

(106) Bohmer, M.; Colby, T.; Bohmer, C.; Brautigam, A.; Schmidt, J.; Bolker, M. Proteomic analysis of dimorphic transition in the phytopathogenic fungus Ustilago maydis. *Proteomics* **2007**, *7* (5), 675–685.

(107) El-Bebany, A. F.; Rampitsch, C.; Daayf, F. Proteomic analysis of the phytopathogenic soilborne fungus Verticillium dahliae reveals differential protein expression in isolates that differ in aggressiveness. *Proteomics* **2010**, *10* (2), 289–303.

(108) Elad, Y.; Williamson, B.; Tudzynski, P.; Delen, N. *Botrytis: Biology, Pathology and Control*; Springer: Dordrecht, 2007.

(109) Mey, G.; Bally, P.; Beffa, R.; González-Fernández, R.; Jorrin-Novo, J. V.; Kaiser, S.; Latorse, M. P.; Schumacher, J.; Tudzynski, P.; Viaud, M. Creation of a collection of Botrytis cinerea T-DNA transformants for pathogenic development and plant defence studies. XVth International Botrytis Symposium, Cadiz, Spain, 2010; p O5.5.

(110) Agrios, G. N. *Plant Pathology*, 5th ed.; Elsevier Academic Press: Amsterdam; Boston, 2005.

(111) Trail, F. For blighted waves of grain: Fusarium graminearum in the postgenomics era. *Plant Physiol.* **2009**, *149* (1), 103–110.