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Proteomic studies of phytopathogenic fungi, oomycetes and their interactions with hosts

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Abstract Proteomics, the systematic analysis of the proteome, is a powerful tool in the post-genomic era. Proteomics studies have examined global changes in proteomes of phytopathogenic fungi, oomycetes and their hosts during compatible or incompatible interactions. This article compiles proteomics reports in order to decipher the molecular mechanisms underlying fungal development (infection-related morphogenesis), fungal or oomycete—host plant interactions, and phytopathogenesis.

Keywords Fungal proteomics · Fungal/Oomycete-plant interaction · Fungal development · Secretome · Virulence differentiation · Oomycetes

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Abbreviations

2-DE/MS	two-dimensional electrophoresis/ mass spectrometry
2D-DIGE/ MS	two-dimensional differential gel electrophoresis/mass spectrometry
MudPIT	multidimensional protein identification technology
LC/MS	liquid chromatography/mass spectrometry
MALDI-TOF	matrix-assisted laser desorption ionisation-time-of-flight
ESI-Q-TOF	electrospray ionisation quadrupole
MS/MS	time-of-flight tandem mass spectrometry
Qq	hybrid quadrupole
nESI	nano electrospray ionisation
IT	ion trap

Introduction

Fungi and oomycetes are the causal agents of many of the world's most serious plant diseases and are unique among the microbial pathogens in being able to breach the intact surfaces of host plants, rapidly establishing infections that can result in significant yield loss in large-scale agricultural production (Soanes et al. 2007). With the completion of the sequencing of 12 phytopathogenic fungal (*Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Fusarium graminearum*, *F. oxysporum*, *F.*

verticillioides, *Magnaporthe oryzae*, *Nectria haematococca*, *Mycosphaerella fijiensis*, *M. graminicola*, *Puccinia graminis*, *Stagonospora nodorum* and *Ustilago maydis*) and two oomycete genomes (*Phytophthora ramorum* and *P. sojae*), the monitoring of global changes in the proteome, referred to as proteomics, is an increasingly attractive method for dissecting the molecular basis of fungal development, fungal-plant interactions, and phytopathogenesis. The term proteomics was coined in 1997 and has been defined as the systematic analysis of the proteome, the protein complement expressed by a genome, cell or tissue (James 1997).

Currently, two gel-based (2-DE and 2D-DIGE) and one non-gel-based (multidimensional liquid chromatography) protein separation techniques coupled with high throughput mass spectrometric tools (MALDI-TOF/TOF, ESI-IT, ESI- triple quadrupole or hybrid TOF MS) are widely used in fungal proteomics. These proteomic methodologies allow scientists to visualise snapshots of the proteins expressed by fungal genomes or by plant genomes under biotic (e.g. fungal and oomycete pathogen attack) and abiotic stresses. In 2-DE, proteins are separated in one-dimension by isoelectric focusing (IEF) and then in orthogonal direction by molecular mass. In 2D-DIGE, protein samples are pre-labelled with fluorescent cyanine dyes (Cy2, Cy3 and Cy 5) prior to IEF. Up to three samples can be run on the same gel, and this permits higher reproducibility of results as compared to 2-DE. To further enhance reproducibility and resolution, a new non-gel-based protein separation technique known as multidimensional protein identification technology (MudPIT) was introduced in proteomics research. In MudPIT, two-dimension liquid chromatography is coupled with ESI-MS/MS, and was first applied to analyse the yeast proteome. Using MudPIT, a total of 1,484 proteins, including low-abundance proteins (transcription factors and protein kinases) were identified from the mid-log phase of *Saccharomyces cerevisiae* (Washburn et al. 2001). Protein separation techniques coupled with mass spectrometry have emerged as influential tools in the field of protein identification and protein complex deconvolution (Figeys et al. 2001). A typical mass spectrometer consists of an ion source, a mass analyser, and a detector. Soft ionisation techniques like MALDI and ESI are used to volatile and ionise large biomolecules, such as peptides and proteins. These ionisation techniques coupled with mass analysers like ion trap (IT), time-of-flight (TOF), quadrupole (Q) or Fourier transform ion cyclotron (FT), provide peptide mass finger prints (PMFs). Introduction of laser-induced and high-energy collision induced dissociation (LID and CID), and in-source decay in reflector mode of mass analyser (reISD) can further analyse these peptide fragments. MALDI is generally coupled with TOF, while ESI can be combined with TOF or separation techniques like liquid chromatography (LC). This allows high-throughput and on-line analysis of peptide mixtures (Ducret et al, 1998; Gatlin et al. 1998). A typical fungal proteomics strategy is illustrated in Fig. 1.

The application of proteomics to phytopathogenic fungi and oomycetes is lagging behind with respect to animals and humans. This review summarises proteomics studies of phytopathogenic fungi, oomycetes and their interactions with hosts (Table 1). These studies have focused on a wide array of research areas, such as fungal/oomycete-plant interactions, fungal development, secretome, virulence, and fungal/oomycete proteins expressed *ex planta* under different conditions.

Fungal-plant/oomycete-plant interactions

Plants have developed an advanced surveillance system to detect invading microbial pathogens like fungi and oomycetes, which has been called innate immunity. Plant innate immunity can be differentiated into basal and resistance (*R*) gene-mediated immunity. Basal immunity is based on recognition of conserved microbial molecules like pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) by pathogen/pattern recognition receptors (PRRs) located at the host plasma membrane (Bent and Mackey 2007). The *R*-gene-mediated immunity follows the gene-for-gene hypothesis, and thus the presence or absence of a single *R* gene confers resistance or susceptibility to a plant in response to a pathogen bearing the corresponding avirulence (*Avr*) gene (Flor 1971). Inducible or active defence mechanisms triggered by PAMPs or AVR proteins mainly involve the oxidative burst, cell wall apposition (papilla formation) at the site of attempted penetration, hypersensitive reaction (HR) cell death, production of antimicrobial metabolites (phytoalexins), and transcriptional activation of pathogenesis-related (PR) proteins.

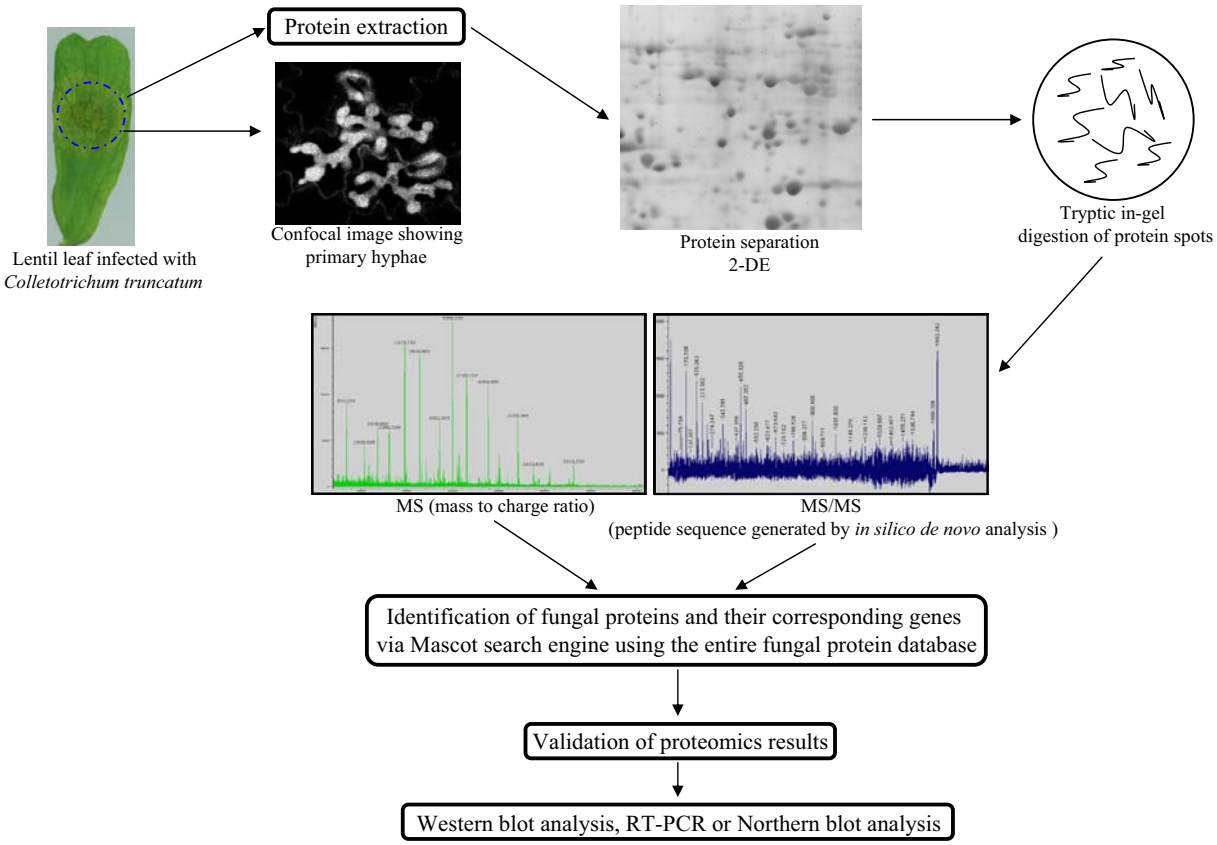

Fig. 1 An illustrative fungal proteomics strategy

These defence responses are mediated by signalling molecules, such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). SA confers resistance to host plants especially against biotrophs whereas JA and ET signalling contributes to resistance against necrotrophs (Ausubel 2005; Chisholm et al. 2006; Dixon and Lamb 1990; Lu et al. 2001). In general, an interaction is incompatible when the plant recognises the invading pathogen early enough through perception of PAMPS or MAMPS by PRRs, or through *R*-gene activation by AVR proteins. This recognition triggers rapid and effective defence responses through signalling pathways involving SA, JA and/or ET. In contrast, an interaction is compatible when the plant responds too late to restrict invasion by the pathogen.

On the basis of mode of infection, fungal and oomycete phytopathogens can be broadly divided into those that kill the host and feed on the contents (necrotrophs), those that require a living host to complete their life-cycle (biotrophs), and those that act as both biotrophs and necrotrophs at different stages of infection (hemibiotrophs). In the case of biotrophs, it is easy to imagine that *R* gene-mediated response and SA signalling can trigger resistance in the host, as the resulting HR response deprives such pathogens of a food source. However, in case of necrotrophs, it seems that this HR response would merely make life easier for the pathogen (Glazebrook 2005).

Proteomics is a potential tool for dissecting molecular mechanisms underlying fungal/oomycete-plant interactions. Proteomic changes in the host plant as it becomes infected or resists the pathogen can be traced back to the interaction between a single host and pathogen gene pair if the genetics of the host-pathogen interaction follows the gene-for-gene model. The resulting biochemical changes are of great interest because they may give insight into critical 'switch points' in the defence-related pathways that could be manipulated to engineer host plants with improved resistance or immunity to the pathogen. This approach may also help to unveil the more

Table 1 Proteomics studies of phytopathogenic fungi and oomycetes

Fungal phytopathogens or their interactions with hosts	Proteomics technique	Principal findings (References)
1. *Magnaporthe oryzae*-rice	2-DE (4-7 p*I*)/N-terminal sequencing	Identified differentially expressed proteins in suspension-cultured rice cells induced by blast fungus and elicitors (Kim et al. 2003).
2. *Magnaporthe oryzae*- rice	2-DE (4-7 p*I*)/MALDI-TOF MS	Identified eight defence-related differentially expressed proteins in rice leaf blades inoculated with rice blast (Kim et al. 2004a).
3. *Magnaporthe oryzae*	2-DE (4.3-7 p*I*)/RP-HPLC/ Edman degradation	Identified five proteins that were induced during appressorium formation (Kim et al. 2004b).
4. *Fusarium verticillioides*-maize	2-DE (3-10 p*I*)/ MALDI-TOF MS & nESI-IT MS/MS	Identified changes in protein patterns in germinating maize embryos in response to infection with the fungus *F. verticillioides* (Campo et al. 2004).
5. *Fusarium graminearum*-wheat	2-DE (4-7 p*I*)/LC-MS/MS	Identified 15 induced proteins from wheat spikelets infected by *F. graminearum*. (Zhou et al. 2005)
6. *Phanerochaete chrysosporium*	2-DE (3-10 p*I*)/ MALDI-TOF	Exogenous addition of vanillin to *P. chrysosporium* shifted the glyoxylate cycle coupled with a short-cut tricarboxylic acid cycle to the normal tricarboxylic acid cycle. Identified up-regulated proteins involved in vanillin metabolism (Shimizu et al. 2005).
7. *Puccinia triticina*-wheat	2-DE (4-8 p*I*)/Qq-TOF MS/MS	Identified 32 up-regulated proteins from wheat leaves inoculated with *Puccinia triticina* (Rampitsch et al. 2006).
8. *Fusarium graminearum*-wheat	2-DE (4-7 p*I*)/LC-MS/MS	Identified 41 differentially regulated proteins in *T. aestivum* spikelets inoculated with *F. graminearum* (compatible interaction) (Zhou et al. 2006)
9. *Botrytis cinerea*	2-DE (5-8 p*I*)/ MALDI-TOF MS & ESI-IT MS/MS	Catalogued mycelial proteome on pH scale 5–8 (Fernández-Acero et al. 2006).
10. *Erysiphe pisi*-pea	2-DE (5-8 p*I*)/MALDI-TOF MS	Identified leaf proteins implicated in powdery mildew resistance (Curto et al. 2006).
11. *Sclerotinia sclerotiorum*	2-DE (4-7 p*I*)/ESI-q-TOF MS/MS	Identified 18 secreted and 95 mycelial proteins (Yajima and Kav 2006).
12. *Ustilago maydis*	2-DE (3-10 p*I*)/MALDI-TOF MS & ESI-MS/MS	Generated proteome reference map to study the changes accompanying the dimorphic transition from budding to filamentous growth at the protein level (Böhmer et al. 2007).
13. *Fusarium graminearum*-wheat	1-DE/LTQ-FT MS	Identified *in vitro* and *in planta* (wheat heads) proteins secreted by *F. graminearum* (*Gibberella zeae*) (Paper et al. 2007).
14. *Curvularia lunata*	1-DE & 2-DE/MALDI-TOF MS/MS	Identified proteins associated with virulence differentiation in *C. lunata* (Xu et al. 2007).
15. *Uromyces appendiculatus*	MudPIT/Tandem MS	Proteome profiling of germinating uredospores (Cooper et al. 2007).
16. *Fusarium oxysporum* f.sp. *lycopersici* (Fol)-tomato	2-DE (3-10 & 6-11 p*I*)/MALDI-TOF MS & LC-QTOF MS/MS	Identified 21 tomato and 7 fungal proteins in the xylem sap of Fol-infected tomato plants (Houterman et al. 2007).
17. *Cladosporium fulvum*-tomato	2-DE (4-6 p*I*)/MALDI-TOF MS LC-QTOF MS/MS	Identified 3 novel fungal secretory proteins viz., CfPhiA, Ecp6 & 7 (Bolton et al. 2008).
18. *Blumeria graminis* f.sp. *hordei*	2-DE (4-7 p*I*)/MALDI-TOF MS/MS	Generated a conidiospore proteome map. Identified proteins involved in carbohydrate, lipid or protein metabolism (Noir et al. 2008).
19. *Fusarium graminearum*	iTRAQ MS/MS	Profiled differentially expressed proteins of *F. graminearum* (*in vitro* mycotoxin inducing versus control conditions) (Taylor et al. 2008).
20. *Fusarium graminearum*-barley	2-DE/LC-MS/MS	Identified barley spikelet proteins associated with resistance to *F. graminearum* (Geddes et al. 2008).
21. *Phanerochaete chrysosporium*	2-DE (3-10 p*I*)/MALDI-TOF MS	Identified differentially expressed mycelial proteins in response to benzoic acid (Matsuzaki et al. 2008).

Table 1 (continued)

Fungal phytopathogens or their interactions with hosts	Proteomics technique	Principal findings (References)
22. *Pyrenophora tritici-repentis*	2-DE/ESI-q-TOF MS/MS	Differentiation of avirulent race 4 and virulent race 5. Identified 63 differentially expressed proteins. Up-regulated protein in race 5 were involved in microbial virulence in other pathosystems, and encompassed the secreted enzymes α-mannosidase and exo-β-1, 3-glucanase, heat-shock and BiP proteins, and various metabolic enzymes (Cao et al. 2009).
23. *Gossypium hirsutum*-cotton	2-DE/LC-MS/MS	Identified pathogen-induced cotton proteins implicated in post-invasion defence responses (PR-proteins and proteins related to oxidative burst), nitrogen metabolism, amino acid synthesis and isoprenoid synthesis (Coumans et al. 2009).
Oomycetes		
24. *Phytophthora infestans*	2-DE (3.5-10 p*I*)	Analysed protein synthesis during 4 developmental stages: hyphae, cysts, germinating cysts, and appressoria (Krämer et al. 1997).
25. *Phytophthora palmivora*	2-DE (4-7 p*I*)/MALDI-TOF MS	Identified stage specific (mycelial, sporangial, zoospore, cyst and germinated cyst) proteins during asexual development (Shepherd et al. 2003).
26. *Aphanomyces euteiches*-barrel medic	2-DE (3-10 p*I*)/MALD-TOF MS	Analysed root proteome *M. truncatula* in response to *A. euteiches* infection. PR-10 proteins displayed higher abundance during infection (Colditz et al. 2004).
27. *Phytophthora infestans*	2-DE (3-10 p*I*)/LC-MS/MS	Identified differentially expressed proteins during cyst germination and appressorium formation (Ebstrup et al. 2005).
28. *Peronospora viciae*-pea	2D-DIGE (3-10 p*I*)/MALDI-TOF MS & ESI-Q-TOF MS/MS	Catalogued host (pea) leaf proteins, which showed alternation in their abundance levels during a compatible interaction with *P. viciae* (Amey et al. 2008).

complex mechanisms underlying activation of plant defence responses against biotrophic, necrotrophic and hemibiotrophic fungal and oomycete phytopathogens. Proteomics can also be employed to decipher molecular processes that occur during the recognition of pathogens by plants (basal defence) irrespective of the eventual outcome of the interaction (Kav et al. 2007).

Host proteins responsive to fungal phytopathogens

The interaction of the hemibiotrophic fungal pathogen *Magnaporthe oryzae* (causal agent of blast disease) and its host plant rice is a seminal paradigm for understanding plant disease, largely because of its great economic importance, but also because of the molecular-genetic tractability of the fungus (Valent and Chumley 1991). In rice with a resistant phenotype, early recognition of the pathogen by the *R*-gene product in the host triggers rapid and effective defence responses, such as generation of reactive oxygen species (ROS), HR cell death, callose deposition, accumulation of phytoalexins and expression of *PR*-genes. The accumulation of PR- proteins like PR-2 (β-1, 3 glucanase), PR-5 (thaumatin-like protein TLP), PR-9 (peroxidase-POX 22.3) and PR-10 (Probenzole inducible protein), receptor-like protein kinase, isoflavone reductase-like protein and a salt-induced protein were found to be induced during compatible and incompatible rice-blast interactions. Isoflavone reductase is implicated in the production of isoflavone phytoalexins in legumes and accumulates in response to pathogen attack, fungal elicitor treatment, and abiotic stresses (Kim et al. 2003, 2004a). Rampitsch et al. (2006) investigated changes in the leaf proteome of wheat infected by the biotrophic leaf rust *Puccinia triticina*. Elongation factor 1β (EF1β), initiation factor 5a (eIF5a), the α-4 subunit of the 20S proteasome and a 14-3-3 protein were identified as induced proteins by 2-DE/MALDI-Q-TOF MS/MS in *P. triticina*-infected wheat leaf proteome. EF1β and eIF5a proteins are involved in the control of protein turnover; however their role in response to pathogen invasion is not clear. The 20S proteasome breaks down unwanted proteins

whereas 14-3-3 proteins have known roles in biotic and abiotic stress responses (Roberts et al. 2002). Fungal proteins like metabolic enzymes (carbohydrate kinase) and structural proteins (α-tubulin and ribosomal protein) were also observed in the leaf proteome of wheat infected by *P. triticina*.

Fusarium head blight (FHB) or scab, caused by the necrotrophic fungus *Fusarium graminearum* is considered to be one of the most destructive diseases of wheat and barley grown in humid and semi-humid climates (McMullen et al. 1997; Parry et al. 1995). A preliminary proteomics study using 2-DE/LC-MS/MS on hexaploid wheat spikelets infected with *F. graminearum*, revealed that proteins with antioxidant function, such as superoxide dismutase (SOD), dehydroascorbate reductase, and glutathione *S*-transferases (GSTs) showed elevation in their abundance 5 days after inoculation, indicating an oxidative burst of H_2O_2 inside the tissues (Zhou et al. 2005). In another study, the authors identified 41 proteins from wheat spikelets infected with *F. graminearum* in a time-course study. Abundance of these proteins was differentially regulated during compatible interactions. Of these 41 proteins, 33 proteins were wheat proteins associated with defence responses (proteins related to oxidative burst, signalling pathway, and PR proteins) and metabolism (glyoxalaseI, N-acetyl glutamate kinase, glutamate dehydrogenase, RubisCO, phosphoglycerate kinase, tryptophan synthase, cysteine synthase, alcohol dehydrogenase, vacuolar invertase I, glyceraldehyde 3-phosphate dehydrogenase, etc.). Antioxidant proteins like ascorbate peroxidase and GST were newly synthesised or more abundant. This indicates that the oxidative burst could potentially be involved in defending wheat spike cells from *F. graminearum*. Increased abundance of the ankyrin repeat protein and 12-oxo-phytodienoic acid reductase suggested that the JA pathway is most likely stimulated and the SA pathway is inhibited during *F. graminearum* infection. Three PR-proteins, PR-2, PR-3, and PR-5, were detected in response to *F. graminearum* infection. Eight fungal proteins possessed putative function as antioxidants and by acquiring carbon through glycolysis in compatible wheat—*F. graminearum* interactions (Zhou et al. 2006). A similar study was conducted on six barley genotypes with varying levels of resistance. Forty-one differentially regulated proteins responsive to *F. graminearum* were identified from spikelets of six barley genotypes. Identified proteins included those associated with the oxidative stress response, such as malate dehydrogenase and peroxidases, and PR-proteins. Elevated abundance of PR-3 or PR-5 might be associated with resistance in barley genotypes (Geddes et al. 2008).

Curto et al. (2006) reported changes in the leaf proteome of two pea genotypes, JI2480 (resistant) and Messire (susceptible), differing in their resistance to *Erysiphe pisi* (biotroph, causal agent of powdery mildew). Using 2-DE/MALDI-TOF/TOF MS/MS, differentially regulated proteins between genotypes (JI2480 and Messire) and treatments (control and infected leaves) were identified. The identified proteins mainly belong to three functional categories: photosynthesis, carbohydrate catabolism and stress/defense responses. These identified proteins corroborate the hypothesis of an increased activity of the energy metabolism in resistant plants to compensate for the cost of constitutive resistance. Although the majority of proteomics studies on host—pathogen interactions have been focused on the leaf proteome, some reports have also been published on other tissues like the embryo, roots and the xylem. Using 2-DE/MALDI-TOF MS or nanospray ion-trap tandem mass spectrometry (nESI-IT MS/MS), Campo et al. (2004) monitored proteomic changes in germinating maize embryos in response to *Fusarium verticillioides*. Enzymes involved in the protection of plant tissues against oxidative damage and detoxification of cytotoxic products, as represented by catalase, SOD and GST showed induction in their abundance levels. In addition, the abundance of translation initiation factor-5A (involved in initiation of protein synthesis), PR-2, and heat-shock proteins (HSPs, also called molecular chaperone) that participate in the protein folding process and stabilisation, were also found to be increased in fungal-infected maize embryos. These proteins could play a role in the maize defence response to fungal infection. During infection, gluconeogenesis (fructose-bisphosphate aldolase) was enhanced while glycolysis (glyceraldehyde 3-phosphate dehydrogenase) repressed. This suggests that increasing level of sucrose in leaf tissue correlates with resistance to fungal pathogens. In another study, the cotton root proteome after infection with the black root rot fungus *Thielaviopsis basicola* was resolved on 2-DE gels to decipher inducible molecular defence mechanisms in a time-course study. Newly synthesised

proteins were then identified using LC-MS/MS. These proteins encompassed defence and stress-related proteins, such as PR-proteins and proteins likely to be involved in the oxidative burst, carbon, and nitrogen metabolism as well as amino acid and isoprenoid syntheses (Coumans et al. 2009). Separating the xylem sap proteome of *Fusarium oxysporum* f. sp. *lycopersici* infected tomato plants by 2-DE and annotation following either MALDI-TOF MS or LC-Q-TOF MS/MS, identified 21 tomato proteins, including PR-1 (a & b), PR-2, PR-9 (peroxidases), polygalacturonase and subtilisin-like protease. In addition to host proteins, seven fungal proteins (Six 1-4, arabinanase, oxidoreductase, serine protease) were detected as well in xylem sap. Six1 (secreted in xylem 1) and arabinanase might be associated with the full virulence of *F. oxysporum* (Houterman et al. 2007).

Host proteins responsive to phytopathogenic oomycetes

For many years, oomycetes were considered to belong to the fungi based on certain morphological similarities. Both fungi and oomycetes are characterised by filamentous vegetative growth, the production of mycelia and formation of spores through asexual and sexual processes. Similarities also exist in infection structures and the mode of infection. However, cell walls of oomycetes primarily consist of cellulose rather than chitin, which is the main component of true fungal cell walls. Furthermore, hyphae of oomycetes are non-septate and are diploid in their vegetative form, which distinguishes them from true fungi. Based on morphological differences and molecular evidence, it was found that oomycetes were related to heterokont algae and plants and thus they were reclassified under the stramenopiles (Barr 1992; Simpson and Roger 2002). Among the oomycetes, *Phytophthora*, *Pythium*, *Peronospora* and *Aphanomyces* are well known pathogens of high economic significance.

The oomycete pathogen *Aphanomyces euteiches* causes a root rot disease of several legumes, including *Pisum sativum* and *Medicago truncatula* (Hagedorn 1989). A comparative study on the *M. truncatula* root proteome led to the identification of proteins, which responded to infection by *A. euteiches*. The root proteome was resolved on 2-DE gels and annotated by LC-MS/MS. The majority of proteins, which exhibited higher abundance, belonged to the PR-10 family, while other proteins were cell wall proteins and enzymes implicated in the phenylpropanoid-isoflavonoid pathway (Colditz et al. 2004). The proteins of the PR-10 family are structurally related to ribonucleases, suggesting these ribonuclease-like proteins might be associated with the cleaving of foreign RNA during pathogen attack (Edreva 2005). Some of these PR-10 proteins were induced by abscisic acid (ABA)-treatment, but not by drought stress, indicating that increased abundance of these proteins might be pathogen-specific. Flavonoids and isoflavonoids are already known to play an important role in plant disease and defence responses (Dixon 2001). In a similar study, Colditz et al. (2005) compared root proteomes of susceptible and resistant lines of *M. truncatula* after *A. euteiches* infection. Proteasomes, N-ethylmaleimide-sensitive fusion attachment proteins and membrane polypeptides were among those that increased in abundance in the resistant line, whereas in the susceptible line enzymes implicated in the flavonoid biosynthesis pathway and primary metabolism like alcohol dehydrogenase and fructose bisphosphate aldolase were more abundant.

Downy mildew caused by *Peronospora viciae* is one of the most common foliar disease of *P. sativum* (Clark and Spencer-Phillips 2000). Using 2D-DIGE, the pea leaf proteome was analysed 4 days after inoculation with *P. viciae*. Eight proteins, which showed elevated abundance in the proteome from infected leaves, were identified using either MALDI-TOF MS or ESI-Q-TOF MS/MS. Among these proteins were disease resistance response protein PI176 (PR-10 family), abscisic acid responsive protein ABR17 (PR-10 family), three photosynthetic proteins, a glycine-rich RNA binding protein and, cytosolic and chloroplastic glyceraldehyde 3-phosphate dehydrogenases. These proteins are implicated in triggering defence responses, signal transduction, protein turnover, and photosynthesis (Amey et al. 2008).

Fungal development

Specific structures or developmental stages (e.g. cyst, asexual spores or conidia, spore germination, appressorium formation, appressorium penetration) of fungal and oomycete pathogens have caught the attention

of plant pathologists. The monitoring of proteomic changes in different structures or during developmental stages will be crucial for identifying molecular determinants of fungal virulence that may be exploited to prevent or control pathogen infections.

The biotrophic fungal pathogen *Uromyces appendiculatus* is the causal agent of rust disease of beans. Using MudPIT couple with tandem mass spectrometry (MS/MS), the proteome from germinating asexual uredospores of this pathogen was surveyed and compared with ungerminated uredospores. This revealed that uredospores require high energy and structural proteins during germination, indicating a metabolic transition from dormancy to germination (Cooper et al. 2007). The dimorphic phytopathogenic fungus *Ustilago maydis* (biotroph, causal agent of smut disease of corn) has been established as a valuable model system to study fungal dimorphism and pathogenicity (Bölker 2001; Feldbrügge et al. 2004; Kämper et al. 2006). The dimorphic transition in corn smut from non-pathogenic budding to pathogenic filamentous growth is intrinsically associated with the switch from a saprotrophic to a pathogenic lifestyle. Filament formation is characterised by highly polarised growth at the tip of a cell and is critical for pathogenic development in *U. maydis*. Therefore, understanding of the underlying molecular mechanism of this morphogenetic process is critical to elucidate the switch to pathogenicity. A proteome reference map of *U. maydis* using 2-DE was constructed, and 250 proteins were identified by MALDI-TOF and ESI-MS/MS from this map. Of these 250, 13 proteins significantly increase in abundance after dimorphic transition from budding to filamentous growth, through induced expression of either the gene encoding bW2/bE1 transcription factor or the gene encoding the small GTP-binding protein Rac1. Among the 13 proteins, three (disulphide isomerase, glutaminase A and a hydrolase) were already known to respond to *b*-locus-induction. Discovery of Rac1- and b- regulated proteins support the hypothesis that filament formation during pathogenic development occurs *via* stimulation of a Rac1-containing signalling module (Böhmer et al. 2007).

Using 2-DE/MALDI-TOF MS, a proteome map was generated from conidiospores of *Blumeria graminis* f. sp. *hordei* (biotroph, causal agent of barley powdery mildew). One hundred and eighty proteins were resolved on this map. The majority of proteins have a predicted function in lipid, carbohydrate or protein metabolism, suggesting that the conidiospore is equipped for the catabolism of storage compounds. This map serves as a platform for the comparative proteomics analyses of further developmental phases of obligate biotrophic phytopathogens. The proteomic features specific to the feeding organs (haustoria), in particular, promise to be a rich source of information on the biotrophic lifestyle (Noir et al. 2008).

Kim et al. (2004b) detected five proteins that were induced during appressorium formation in *M. oryzae* using 2-DE. Four of them were identified by reverse phase-high performance liquid chromatography (RP-HPLC)-Edman degradation as 20S proteasome α subunits (MgP1 and MgP5), scytalone dehydratase (SCD) and serine carboxypeptidase Y (CPY). Proteasomes play important roles in protein turnover in both the cytosol and the nucleus. They selectively degrade intracellular unwanted proteins through the ubiquitin-proteasome pathway (Baumeister et al. 1998). SCD is a key enzyme of the melanin biosynthetic pathway, as it catalyses dehydration steps (i.e. from scytalone to trihydroxynaphthalene and vermelone to trihydroxynaphthalene) (Basarab et al. 1999; Jordan et al. 2000a, b). In phytopathogenic fungi like *M. oryzae*, SCD is essential for appressorium maturation and the ability of the appressorium to build up turgor pressure needed for the infection peg to pierce the host leaf surface and thus infect the host. CPY is localised in vacuoles and implicated in cellular homeostasis.

Phytophthora infestans is an oomycete plant pathogen that caused the devastating late blight epidemics of potato in the1840s in Europe. Using 2-DE, Krämer et al. (1997) analysed protein synthesis during four distinct developmental stages of the fungus, such as hyphae, cysts, germinating cysts and appressoria, and noticed that the majority of changes in protein synthesis occurred during cyst germination and development of the germ tubes. Several proteins were newly synthesised at the stage of appressorium formation. In another similar study, a 2-DE/LC-MS/MS-based proteomics approach was employed to investigate proteomic changes during cyst germination and appressorium formation in *P. infestans*. Proteins implicated in protein synthesis (e.g. a DEAD box RNA helicase), amino acid metabolism, energy metabolism and ROS scavenging were found to be affected by germination of cysts and appressorium formation (Ebstrup et al. 2005). Shepherd et al.

(2003) used 2-DE to analyse stage-specific proteins from *Phytophthora palmivora* (causal agent of black pod disease of cocoa) and found that approximately 1% of proteins were stage-specific among the five stages, i.e. mycelia, sporangia, zoospores, cysts and germinated cysts.

Secretome

Many phytopathogenic fungi secrete a large number of proteins, collectively known as the secretome, to accommodate their parasitic lifestyle. Some of these secretory proteins enable phytopathogenic fungi and oomycetes to invade, penetrate and colonise the plant cells. Therefore, components of the secretome like effectors are crucial for the pathogenicity and virulence of phytopathogens. Effectors are defined as proteins that manipulate biochemical, physiological, and morphological processes in their host plants, thereby facilitating infection (virulence factors or toxins) and/or triggering defence responses (avirulence factors or elicitors) (Bent and Mackey 2007). Avirulence proteins encoded by effector genes of fungi and oomycetes are usually small (<150 amino acids) hydrophobic proteins. Some of these proteins are active in the apoplast where they may interfere with host plant defence processes, e.g. by inhibiting plant proteases and lytic enzymes. Other fungal proteins enter host cells, albeit the mechanism of entry of these effectors is still unknown. However, the mechanism of translocation of oomycete effector proteins into the host cytoplasm has recently been elucidated. Genetic mapping of avirulence genes led to the cloning of four effector genes, *viz. Avrb-1* from *P. sojae* (Shan et al. 2004), *Avr3a* from *P. infestans* (Armstrong et al. 2005), and *ATR1* (Rehmany et al. 2005) and *ATR13* (Allen et al. 2004) from *Hyaloperonospora arabidopsidis*. Sequence analyses of these genes revealed that the effectors encoded by these genes have two common motifs, RXLR (arginine, any amino acid, leucine, arginine) and dEER [aspartate (less conserved), glutamate, glutamate, arginine] (Rehmany et al. 2005; Birch et al. 2006; Tyler et al. 2006). The discovery of the RXLR-dEER motifs has led to the identification of several other effectors from *Phytophthora* spp., namely Avr4/6, Avr1a, Avr3a, Avr4 and AvrBIb1. These effectors with RXLR-dEER motifs are secreted into the apoplast where they target the proteins for endocytosis by host cells (Whisson et al. 2007). The main function of these fungal and oomycete effectors is to suppress the host signal transduction pathways that mediate defence responses.

Several approaches have been employed to characterise the secretome of fungal and oomycete phytopathogens. Proteomics analyses using the 2-DE/MS-based approach can detect polypeptides in culture filtrates or fluids like xylem sap from infected plants (Paper et al. 2007; Houterman et al. 2007). However, protein identification has remained elusive for pathogens for which genome sequence information is not available. Other approaches exploit *in silico* algorithms and the signal sequence trapping. Biocomputational algorithms are used to scan N-terminal signal peptides in predicted proteins from whole-genome sequences or expressed sequence tags (ESTs). Using this approach, dozens of secreted proteins were identified from *P. infestans* (Torto et al. 2003; Tian et al. 2004), *Colletotrichum higginsianum* (Kleemann et al. 2008), and *Venturia inaequalis* (Brown et al. 2009). The signal sequence trap is a yeast-based genetic screen for signal peptide encoding cDNA. It is based on the complementation of a reporter gene lacking a signal peptide (Klein et al. 1996). This approach has been applied successfully to identify secreted proteins from *P. sojae* (Lee et al. 2006), *U. fabae* (Link and Voegele 2008), and *Colletotrichum graminicola* (Krijger et al. 2008).

The leaf mould pathogen *Cladosporium fulvum* and its host tomato have been studied in great detail with regard to gene-for-gene (*Avr*/*R*) interactions. This biotrophic fungal pathogen secretes several effectors into the apoplast during tomato leaf colonisation. Therefore, apoplastic extracts from *C. fulvum*-colonised tomato leaves can serve as an important resource for the discovery of effectors. Using 2-DE, Bolton et al. (2008) visualised the apoplastic proteome during compatible and incompatible *C. fulvum*-tomato interactions and identified three novel effectors by MALDI-TOF MS and LC MS/MS, *viz*. a CfPhiA (*C. fulvum* PhialidesA) and two extracellular proteins (Ecp6 and Ecp7). Ecp6 contains three chitin-binding lysine motifs (LysM). Heterologous expression and gene silencing have demonstrated that Ecp6 is a virulence factor. Ecp6 may act as a functional homologue of chitin binding Avr4 or act as a stealth factor by shielding fungal hyphae through sequester-

ing chitin monomers or oligomers to avoid recognition by the host plant. CfPhiA showed homology with the PhiA protein from *Aspergillus nidulans*, which is important for phialide and conidium development (Melin et al. 2003). *Ecp7* encodes a 11 kDa cysteine-rich protein with an even number of cysteine residues considered to be indicative for disulphide bridges. This configuration would protect the stability and function of Ecp7 in the proteinase-rich apoplast environment of the host. *Cladosporium fulvum* resistance genes of tomato encode R-proteins (receptor-like proteins) containing leucine-rich repeat—transmembrane domains (LRR-TM). These R-proteins recognise *C. fulvum* effectors like AVR2, 4, 5, and 9 secreted into the leaf apoplast (apoplastic effectors). This recognition triggers host cell defence responses, including HR cell death. Other apoplastic effectors function as counter-defence mechanisms against host hydrolytic enzymes, usually PR-proteins, such as PR-2, chitinases (PR-3, 4, 8 and 11), and proteases (PR-6 and 7). Apoplastic effector molecules also include the large class of small cysteine-rich proteins containing typical elicitins, AVR proteins, and the necrosis- and ethylene-inducing (NEP1)-like protein family as well as fungal cell wall glycoproteins, such as transglutaminases or the cellulose-binding elicitor lectin from oomycete pathogens (Kamoun 2005).

In contrast to the apoplastic AVR/R interaction, many other fungal and oomycete effectors enter into the cells (cytoplasmic effectors), where they interact with cytoplasmic R-proteins (NBS-LRR, nucleotide binding site-leucine-rich repeat; PK, protein kinases). *Arabidopsis* R-proteins RPP1, 5, 8 and 13 that interact with AVR proteins AVR-RPP1, AVR-RPP5, AVR-RPP8, and AVR-RPP13 of *Peronospora parasitica*, and rice R-proteins Pi-ta, Pib and Piz-t that interact with AVR proteins AVR-Pita, AVR-Pib, and AVR-Pizt of *M. oryzae* represent some of the NBS-LRR class R-proteins. All of these R-proteins are located in the cytoplasm where they interact with the corresponding cytoplasmic effectors.

Another class of fungal secretory proteins includes hydrolytic enzymes particularly cell wall-degrading enzymes (CWDEs). Plant cell walls are the first major point of contact between the plant and the pathogen. Cell walls consist mainly of polysaccharides like cellulose, hemicelluloses, and pectin, together with structural proteins such as hydroxyproline-rich glycoprotein extensin. For successful penetration and colonisation of host plants, pathogens must be able to degrade these cell walls. To accomplish this, fungal and oomycete pathogens secrete an array of polysaccharide-degrading enzymes, including exo- and endo-polygalacturonases, pectin methylesterases, pectin lyases and pectate lyases, acetyl esterases, xylanases and a variety of endoglucanases that cleave cellulose, xyloglucan and other glucans (Lebeda et al. 2001). In response to CWDEs, plants deploy inhibitory proteins to inactivate and degrade CWDEs, e.g. polygalacturonase-inhibiting proteins, pectin methylesterase inhibitor, pectin lyase inhibitor protein, xylanase inhibitor protein, and xyloglucan endoglucanase inhibiting protein. The exponential increase in genomic data available for plants and microbes has made it possible to survey CWDEs and their inhibitor proteins implicated in plant-pathogen interactions. Proteomics analyses will contribute greatly in this regard. So far, only few studies have exploited this approach to identify and characterise CWDEs and their inhibitors. *Sclerotinia sclerotiorum* is a necrotrophic phytopathogenic ascomycete, capable of infecting over 400 dicotyledonous herbaceous plant species (Boland and Hall 1994). To better comprehend its life-cycle and ability to infect susceptible plants, a proteome-level study of the secretome was conducted using 2-DE/ESI-Q-TOF MS/MS. Among 18 identified proteins, many were implicated in the maceration of components found in the outer surfaces of susceptible plants, thereby facilitating invasion and colonisation of the plant cells by the invading fungus (Yajima and Kav 2006). Among those CWDEs were endo- and exo-polygalacturonases, pectin methyl esterases, alpha-L-arabinofuranosidase, cellobiohydrolase, acid proteases and aspartyl proteinase. A high-throughput MS/MS (LTQ/FT) was used to identify proteins secreted by *F. graminearum in vitro* and *in planta* (wheat head). *In planta* proteins identified include degradative enzymes (hydrolases, oxidoreductases, esterases, and proteases) and small non-enzymatic proteins (Paper et al. 2007).

Virulence

The most effective disease management tool against fungal and oomycete pathogens is the development and deployment of highly resistant crop varieties. This approach has been successfully used in host-pathogen systems governed by the gene-for-gene

interaction, where the identification and use of *R*-genes are relatively simple because of clearly distinguishable compatible and incompatible interactions. In contrast, identifying effective *R*-genes in host plants against some necrotrophic pathogens has been elusive, primarily because the outcome of interactions between the host and different isolates of necrotrophic pathogens varies quantitatively rather than in discrete terms. Xu et al. (2007) exploited 1-DE and 2-DE/MALDI-TOF/TOF MS/MS approaches to unveil the mechanism underlying variations in virulence in *Curvularia lunata* (necrotroph, causal agent of leaf spot disease of maize). Twenty differentially regulated proteins were identified in the virulent strain CX-3 and the relatively less virulent strain DD-60 proteomes of *C. lunata*. Three proteins were unique to strain CX-3 whereas the remaining were present in higher abundance in this virulent strain. These proteins were associated with energy metabolism, stress tolerance (including HSPs) and signal transduction, and it was suggested that the interaction of these proteins may contribute to the virulence phenotype. The presence of HSPs like HSP70 was believed to be beneficial by improving the pathogen's tolerance for high temperature stress during penetration of host plants in hot summer that promote this disease. In addition, HSPs may fold around other important proteins damaged by stress thereby protecting them from being denatured. Two proteins, *viz*. UBSR1 and Brn1 were also more abundant in the virulent strain CX-3, and were identified as a transcriptional regulator in siderophore biosynthesis and an enzyme involved in melanin biosynthesis, respectively. Increased accumulation of siderophores in mycelium improves a pathogen's competitiveness for iron absorption from surrounding environments, which then may result in a lack of available iron for plant growth. The *Brn1* gene encodes 1, 3, 8- trihydroxynaphthaphthalene reductase (T3HNR), a key enzyme of melanin biosynthesis, and is essential for conidial development. It was speculated that the increased abundance of HSPs, UBSR1, and in particular Brn1 proteins in CX-3 may contribute to the higher virulence in this and other virulent strains.

Pyrenophora tritici-repentis, causal agent of tan spot of wheat, produces host-specific toxins that determine compatibility between certain races of the pathogen and sensitive wheat varieties (Lamari et al. 1995). Using 2-DE/ESI-Q-TOF, the avirulent race 4 could be differentiated from the virulent race 5 of this pathogen. The proteins implicated in virulence of race 5 included secreted enzymes α-mannosidase and exo-β-1, 3-glucanase, heat-shock and BiP proteins, and various metabolic enzymes (Cao et al. 2009).

Botrytis cinerea is an ascomycetous phytopathogenic fungus, causing disease on a number of crops. This species is characterised by a high level of variability in virulence among isolates, as well as phenotypic instability (Beever and Weeds 2004). A 2-DE/MALDI-TOF MS or ESI IT MS/MS-based approach was used to characterise fungal virulence factors of two isolates of *B. cinerea* (Fernández-Acero et al. 2006). Twenty-eight protein spots were identified, 17 of which corresponded to malate dehrogenase (MDH), and were abundant in the more virulent isolate. Four proteins were identified as glyceraldehyde-3-phosphate dehydrogenases (GADPH), and were only found in the proteome of the more virulent strain. MDH is a ubiquitous key regulatory enzyme of the energetic metabolism *via* the tricarboxylic acid pathway (TCA). It can catalyse the reversible conversion of oxaloacetate, an oxalic acid precursor, which is considered to be a pathogenicity factor in *B. cinerea*. In addition, acidification of the environment by oxalic acid would lead to the production of *B. cinerea* toxins, such as botrydial and dihydrobotrydial. GAPDH is reported to be a virulence factor for a vast variety of microbial pathogens. Finally, one protein, again only found in the more virulent isolate, was identified as a cytosolic cyclophilin, which also functions as a virulence factor in *B. cinerea* (Fernández-Acero et al. 2006).

Ex planta expressed proteins

Apart from the aforementioned studies, some proteomics research has been focused on *in vitro* fungal responses upon exogenous addition of substrates that mimic host target compounds. Lignin is a random, heterogeneous phenylpropanoid polymer and is one of the most recalcitrant biomaterials on earth (Sarkanen and Ludwig 1971; Crawford 1981). Basidiomycetes are the only known microorganisms with the ability to degrade lignin. They have been suspected of possessing a unique metabolic system for degradation of a variety of aromatic compounds (Shimizu et al. 2005). Proteomics studies to monitor *ex planta* fungal, specifically basidiomycete responses to exogenous

addition of substrates, such as vanillin and benzoic acid (BA) will be instrumental in understanding the mechanisms underpinning the ability of these organisms to degrade a wide variety of aromatic compounds like lignin. Vanillin and BA are plant phenolic compounds derived from the phenylpropanoid pathway. Shimizu et al. (2005) used the proteomic differential display technique to monitor cellular responses of *Phanerochaete chrysosporium* (causal agent of white-rot disease) exposed to vanillin, one of the key intermediates found during lignin biodegradation. The addition of vanillin to *P. chrysosporium* enhanced glucose consumption but inhibited fungal growth, indicating that vanillin is not a nutrient substrate, but acts rather as a chemical stress on fungal cells. Upon addition of vanillin to *P. chrysosporium*, the homogentisate 1,2-dioxygenase, 1,4-benzoquinone reductases, aldehyde dehydrogenase, and aryl-alcohol dehydrogenase increased in abundance, all of which have been implicated in vanillin metabolism. The most interesting observation noted upon exogenous addition of vanillin was the dramatic shift of the glyoxylate cycle coupled with a short-cut TCA pathway to the normal tricarboxylic acid cycle, which seemed to trigger heme biosynthesis and energy production. In another study, intracellular processes of *P. chrysosporium* upon exogenous addition of BA were investigated using 2-DE/MS. Enzymes like aryl-alcohol dehydrogenase, arylaldehyde dehydrogenase, and cytochrome P450s showed elevated abundance, suggesting that these enzymes are crucial for BA metabolism. BA is known to have an inhibitory effect on fungal growth (Matsuzaki et al. 2008).

Fusarium graminearum synthesises trichothecene mycotoxins during host plant attack to facilitate spread of the pathogen in wheat and maize which results in reduced grain quantity and quality (Desjardins et al. 1996; Harris et al. 1999). To better understand the fungal activities and pathways that are active during mycotoxin production, a global fungal proteome under *in vitro* conditions conducive to trichothecene production was compiled. The 2-DE gels are generally limited to resolving abundant proteins; proteins of low abundance, of high hydrophobicity, extreme p*I* or high molecular weight, which are therefore rarely identified. To circumvent these bottlenecks of gel-based protein separation techniques, Taylor et al. (2008) employed iTRAQ (isobaric tags for relative and absolute quantification) a non-gel-based quantitative shotgun proteomics approach to identify proteins and quantify their relative abundance between several samples. A number of induced proteins involved in virulence, including secreted proteins were identified. The trichothecene C-3 acetyltransferase protein involved in trichothecene biosynthesis was one of the proteins that significantly increased in abundance. In addition to increases in protein abundance, this approach also allowed scientist to identify proteins that had become scarcer, and which were enzymes in the primary metabolism, protein chaperones, or proteins involved with the cellular translational machinery. *In vitro* conditions can provide an alternative platform to conduct fungal proteomics studies in response to substrates, which mimic fungal development and plant infection.

Concluding remarks

With the advent of high-throughput sequencing technologies, the number of fungal and oomycete genomes sequenced has been increasing rapidly. The increasing availability of complete genome data and the introduction of advanced protein identification technologies like MALDI-TOF and ESI-MS/MS have fuelled the potential in fungal proteomics to monitor global changes in proteomes during fungal-plant interactions (compatible or incompatible), fungal development, and phytopathogenesis. This will enable scientists to better understand the biochemical mechanisms underlying plant defence responses, the role of fungal effectors in the establishment of disease, and the structural and regulatory components involved in spore germination, appressorium morphogenesis, penetration, and haustorial formation. However, there still remain challenges in proteomics technology, especially in protein separation and analysis. A major drawback in gel-based proteomics techniques is reproducibility in protein spots on gels. Efforts have been made to overcome this shortcoming. As an alternative without resolving spots on gels, protein samples can be in-solution digested and peptide fragments obtained can then be separated using liquid chromatography (LC) followed by tandem mass spectrometry (MS/MS). There are many complementary technologies, such as protein arrays, the yeast two-hybrid system, phase display antibody libraries, surface-enhanced laser

desorption and ionisation, that are being developed and either alone or in combination will undoubtedly be effective in fungal proteomics and functional or structural genomics-based approaches.

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References

Allen, R. L., Bittner-Eddy, P. D., Grenvitte-Briggs, L. J., Meitz, J. C., Rehmany, A. P., Rose, L. E., & Beynon, J. L. (2004). Host-parasite coevolutionary conflict between Arabidopsis and downy mildew. *Science, 306*, 1957–1960.

Amey, R. C., Schleicher, T., Slinn, J., Lewis, M., Macdonald, H., Neill, S. J., et al. (2008). Proteomic analysis of a compatible interaction between *Pisum sativum* (pea) and the downy mildew pathogen *Peronospora viciae*. *European Journal of Plant Pathology*, *122*, 41–55.

Armstrong, M. R., Whisson, S. C., Pritchard, L., Bos, J. I. B., Venter, E., Avrova, A. O., et al. (2005). An ancestral oomycete locus contains late blight avirulence gene *Avr3a*, encoding a protein that is recognised in the host cytoplasm. *Proceedings of the National Academy of Sciences USA, 102*(21), 7766–7771.

Ausubel, F. M. (2005). Are innate immune signaling pathways in plants and animals conserved? *Nature Immunology*, *6*, 973–979.

Barr, D. J. S. (1992). Evolution and kingdoms from the perspective of a mycologist. *Mycologia*, *84*, 1–11.

Basarab, G. S., Jordan, D. B., Gehret, T. C., Schwartz, R. S., & Wawrzak, Z. (1999). Design of scytalone dehydratase inhibitors as rice blast fungicides: derivatives of norephedrine. *Bioorganic and Medicinal Chemistry Letters*, *9*, 1613–1618.

Baumeister, W., Walz, J., Zühl, F., & Seemüller, E. (1998). The proteasome: paradigm of a self-compartmentalizing protease. *Cell*, *92*, 367–380.

Beever, R. E., & Weeds, P. L. (2004). Taxonomy and genetic variation of *Botrytis* and *Botryotinia*. In Y. Elad, B. Williamson, P. Tudzynski & N. Delan (Eds.), *Botrytis: Biology, pathology and control* (pp. 29–52). Dordrecht: Kluwer.

Bent, A., & Mackey, D. (2007). Elicitors, effectors and R genes: the new paradigm and a lifetime supply of questions. *Annual Review of Phytopathology*, *45*, 399–436.

Birch, P. R., Rehmany, A. P., Pritchard, L., Kamoun, S., & Beynon, J. L. (2006). Trafficking arms: Oomycete effectors enter host plant cells. *Trends in Microbiology, 14*, 8–11.

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

Boland, G. J., & Hall, R. (1994). Index of host plants of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology*, *16*, 93–108.

Bölker, M. (2001). *Ustilago maydis*—a valuable model system for the study of fungal dimorphism and virulence. *Microbiology*, *147*, 1395–1401.

Bolton, M. D., van Esse, H. P., Vossen, J. H., de Jonge, R., Stergiopoulos, I., Stulemeijer, I., et al. (2008). The novel *Cladosporium fulvum* lysin motif effector Ecp6 is a virulence factor with orthologues in other fungal species. *Molecular Microbiology*, *69*(1), 119–136.

Brown, J. K., Mesarich, C. H., Rees-George, J., Cui, W., Fitzgerald, A., Win, J., et al. (2009). Candidate effector gene identification in the ascomycete fungal phytopathogen *Venturia inaequalis* by expressed sequence tag analysis. *Molecular Plant Pathology*, *10*(3), 431–448.

Campo, S., Carrascal, M., Coca, M., Abián, J., & Segundo, B. S. (2004). The defense response of germinating maize embryos against fungal infection: a proteomics approach. *Proteomics*, *4*, 383–396.

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

Chisholm, S. T., Coaker, G., Day, B., & Staskawicz, B. J. (2006). Host—microbe interactions: shaping the evolution of the plant immune response. *Cell*, *124*, 803–814.

Clark, J. S. C., & Spencer-Phillips, P. T. N. (2000). Downy Mildews. In J. Lederberg, M. Alexander, B. R. Bloom, D. Hopwood, R. Hull, B. H. Inglearski, A. I. Laskia, S. G. Oliver, M. Schaechter & W. C. Summers (Eds.), *Encyclopaedia of microbiology* (Vol. 2, pp. 117–129). San Diego: Academic.

Colditz, F., Nyamsuren, O., Niehaus, K., Eubel, H., Braun, H. P., & Krajinski, F. (2004). Proteomic approach: identification of *Medicago truncatula* proteins induced in roots after infection with the pathogenic oomycete *Aphanomyces euteiches*. *Plant Molecular Biology*, *55*, 109–120.

Colditz, F., Braun, H. P., Jacquet, H., Niehaus, K., & Krajinski, F. (2005). Proteomic profiling unravels insights into the molecular background underlying increased *Aphanomyces uteiches* tolerance of *Medicago truncatula*. *Plant Molecular Biology*, *59*, 387–406.

Cooper, B., Neelam, A., Campbell, K. B., Lee, J., Liu, G., Garrett, W. M., et al. (2007). Protein accumulation in the germinating *Uromyces appendiculatus* uredospore. *Molecular Plant-Microbe Interactions*, *20*(7), 857–866.

Coumans, J. V. F., Poljak, A., Raftery, M. J., Backhouse, D., & Pereg-Gerk, L. (2009). Analysis of cotton (*Gossypium hirsutum*) root proteomes during a compatible interaction with the black root rot fungus. *Proteomics*, *9*, 335–349.

Crawford, R. L. (1981). *Lignin biodegradation and transformation*. New York: Wiley.

Curto, M., Camafeita, E., Lopez, J. A., Maldonado, A. M., Rubiales, D., & Jorrín, J. V. (2006). A proteomic approach to study pea (*Pisum sativum*) responses to powdery mildew (*Erysiphe pisi*). *Proteomics*, *6*, S163–S174.

Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., Buechley, G., et al. (1996). Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. *Molecular Plant-Microbe Interactions*, *9*, 775–781.

Dixon, R. A. (2001). Natural products and disease resistance. *Nature*, *411*, 843–847.

Dixon, R. A., & Lamb, C. J. (1990). Molecular communication in interactions between plant and microbial pathogens. *Annual Review of Plant Physiology and Plant Molecular Biology*, *41*, 339–367.

Ducret, A., van Oostveen, I., Eng, J. K., Yates, J. R., III, & Aebersold, R. (1998). High throughput protein characterization by automated reverse-phase chromatography/electrospray tandem mass spectrometry. *Protein Science*, *7*, 706–719.

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

Edreva, A. (2005). Pathogenesis-related proteins: research progress in the last 15 years. *General and Applied Plant Physiology*, *31*, 105–124.

Feldbrügge, M., Kämper, J., Steinberg, G., & Kahmann, R. (2004). Regulation of mating and pathogenic development in *Ustilago maydis*. *Current Opinion in Microbiology*, *7*, 666–672.

Fernández-Acero, F. J., Jorge, I., Calvo, E., Vallejo, I., Carbú, M., Camafeita, E., et al. (2006). Two-dimensional electrophoresis protein profile of the phytopathogenic fungus *Botrytis cinerea*. *Proteomics*, *6*, S88–S96.

Figeys, D., Linda, D., McBroom, L. D., & Moran, M. F. (2001). Mass spectrometry for the study of protein–protein interactions. *Methods*, *24*, 230–239.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology*, *9*, 275–296.

Gatlin, C. L., Kleemann, G. R., Hays, L. G., Link, A. J., & Yates, J. R., III. (1998). Protein identification at the low femtomole level from silver stained gels using a new fritless electrospray interface for liquid chromatography microspray and nanospray mass spectrometry. *Analytical Biochemistry*, *263*, 93–101.

Geddes, J., Eudes, F., Laroche, A., & Selinger, B. (2008). Differential expression of proteins in response to the interaction between the pathogen *Fusarium graminearum* and its host, *Hordeum vulgare*. *Proteomics*, *8*, 545–554.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, *43*, 205–227.

Hagedorn, D. J. (1989). Aphanomyces root rot. In Compendium of Pea Diseases, American Phytopathological Society Press, St. Paul, MN

Harris, L. J., Desjardins, A. E., Plattner, R. D., Nicholson, P., Butler, G., Young, J. C., et al. (1999). Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. *Plant Disease*, *83*, 954–960.

Houterman, P. M., Speijer, D., Dekker, H. L., De Koster, C. G., Cornelissen, B. J. C., & Rep, M. (2007). The mixed xylem sap proteome of *Fusarium oxysporum*-infected tomato plants. *Molecular Plant Pathology*, *8*, 215–221.

James, P. (1997). Protein identification in the post-genome era: the rapid rise of proteomics. *Quarterly Reviews of Biophysics*, *30*, 279–331.

Jordan, D. B., Basarab, G., Steffens, J. J., Schwartz, R. S., & Doughty, J. G. (2000a). Tight-binding inhibitors of scytalone dehydratase: effects of site-388 directed mutations. *Biochemistry*, *39*, 8593–8602.

Jordan, D. B., Zheng, Y. J., Locket, B. A., & Basarab, G. S. (2000b). Stereochemistry of the Enolization of scytalone by scytalone dehydratase. *Biochemistry*, *39*, 2276–2282.

Kamoun, S. (2005). A catalogue of the effector secretome of plant pathogenic oomycetes. *Annual Review of Phytopathology*, *44*, 1–20.

Kämper, J., Kahmann, R., Bölker, M., Ma, L. J., Brefort, T., Saville, B. J., et al. (2006). Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature*, *444*, 97–101.

Kav, N. V. N., Srivastava, S., Yajima, W., & Sharma, N. (2007). Application of proteomics to investigate plant-microbe interactions. *Current Proteomics*, *4*, 28–43.

Kim, S. T., Cho, K. S., Yu, S., Kim, S. G., Hong, J. C., Han, C., et al. (2003). Proteomic analysis of differentially expressed proteins induced by rice blast fungus and elicitor in suspension-cultured rice cells. *Proteomics*, *3*, 2368–2378.

Kim, S. T., Kim, S. G., Hwang, D. H., Kang, S. Y., Kim, H. J., Lee, B. H., et al. (2004a). Proteomic analysis of pathogen-responsive proteins from rice leaves induced by rice blast fungus, *Magnaporthe grisea*. *Proteomics*, *4*, 3569–3578.

Kim, S. T., Yu, S., Kim, S. G., Kim, H. J., Kang, S. Y., Hwang, D. H., et al. (2004b). Proteome analysis of rice blast fungus (*Magnaporthe grisea*) proteome during appressorium formation. *Proteomics*, *4*, 3579–3587.

Kleemann, J., Takahara, H., Stüber, K., & O'Connell, R. (2008). Identification of *Colletotrichum higginsianum* by analysis of expressed sequence tags. *Microbiology*, *154*, 1204–1217.

Klein, R. D., Gu, Q. M., Goddard, A., & Rosenthal, A. (1996). Selection for genes encoding secreted proteins and receptors. *Proceedings of the National Academy of Sciences USA, 93*, 7108–7113.

Krämer, R., Freytag, S., & Schmelzer, E. (1997). *In vitro* formation of infection structures of *Phytophthora infestans* is associated with synthesis of stage specific polypeptides. *European Journal of Plant Pathology*, *103*, 43–53.

Krijger, J., Horbach, R., Behr, M., Schweizer, P., Deising, H. B., & Wirsel, S. G. R. (2008). The yeast signal trap identifies secreted proteins of the hemibiotrophic corn pathogen *Colletotrichum graminicola*. *Molecular Plant-Microbe Interactions*, *10*, 1325–1336.

Lamari, L., Sayoud, R., Boulif, M., & Bernier, C. C. (1995). Identification of a new race in *Pyrenophora tritici-repentis*: Implications for the current pathotype classification system. *Canadian Journal of Plant Pathology*, *17*, 312–318.

Lebeda, A., Luhova, L., Sedlarova, M., & Jancova, D. (2001). The role of enzymes in plant–fungal pathogens interactions. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, *108*, 89–111.

Lee, S. J., Kelley, B. S., Damasceno, C. M. B., John, B. S., Kim, B. S., Kim, B. D., et al. (2006). A functional screen to characterize the secretomes of eukaryotic pathogens and their hosts in planta. *Molecular Plant-Microbe Interactions*, *19*, 1368–1377.

Link, T., & Voegele, R. T. (2008). Secreted proteins of *Uromyces fabae*: similarities and stage specificity. *Molecular Plant Pathology*, *9*, 59–66.

Lu, M., Tang, X., & Zhou, J. M. (2001). Arabidopsis NHO1 is required for general resistance against Pseudomonas bacteria. *Plant Cell*, *13*, 437–447.

McMullen, M., Jones, R., & Gallenburg, D. (1997). Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Disease*, *81*, 1340–1348.

Matsuzaki, F., Shimizu, M., & Wariishi, H. (2008). Proteomic and metabolomic analyses of the white-rot fungus *Phanerochaete chrysosporium* exposed to exogenous benzoic acid. *Journal of Proteome Research*, *7*, 2342–2350.

Melin, P., Schnürer, J., & Wagner, E. G. H. (2003). Characterization of *phiA*, a gene essential for phialide development in *Aspergillus nidulans*. *Fungal Genetics and Biology*, *40*, 234–241.

Noir, S., Colby, T., Harzen, A., Schmidt, J., & Panstruga, R. (2008). A proteomic analysis of powdery mildew (*Blumeria graminis* f.sp. *hordei*) conidiospores. *Molecular Plant Pathology*, *10*(2), 223–236.

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

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals—a review. *Plant Pathology*, *44*, 207–238.

Rampitsch, C., Bykova, N. V., McCallum, B., Beimcik, E., & Ens, W. (2006). Analysis of the wheat and *Puccinia triticina* (leaf rust) proteomes during a susceptible host-pathogen interaction. *Proteomics*, *6*, 1897–1907.

Rehmany, A. P., Gordon, A., Rose, L. E., Allen, R. L., Armstrong, M. R., Whisson, S. C., et al. (2005). Differential recognition of highly divergent downy mildew avirulence gene alleles by *RPP*1 resistance genes from two *Arabidopsis* lines. *Plant Cell*, *17*, 1839–1850.

Roberts, M. R., Salinas, J., & Collinge, D. B. (2002). 14-3-3 proteins and the response to abiotic and biotic stress. *Plant Molecular Biology*, *50*, 1031–1039.

Sarkanen, K. V., & Ludwig, C. H. (1971). *Lignin: Occurrence, formation, structure, and reactions*. New York: Wiley.

Shan, W., Cao, M., Leung, D., & Tyler, B. M. (2004). The *Avr1b* locus of *Phytophthora sojae* encodes an elicitor and a regulator required for avirulence on soybean plants carrying resistance gene *Rps*1b. *Molecular Plant Microbe Interactions*, *17*, 394–403.

Shepherd, S. J., Van West, P., & Gow, N. A. R. (2003). Proteomic analysis of asexual development of *Phytophthora palmivora*. *Mycological Research*, *107*, 395–400.

Shimizu, M., Yuda, N., Nakamura, T., Tanaka, H., & Wariishi, H. (2005). Metabolic regulation at the tricarboxylic acid and glyoxylate cycles of the lignin-degrading basidiomycetes *Phanerochaete chrysosporium* against exogenous addition of vanillin. *Proteomics*, *5*, 3919–3931.

Simpson, A. G. B., & Roger, A. J. (2002). Eukaryotic evolution: Getting to the root of the problem. *Current Biology*, *12*, R691–R693.

Soanes, D. M., Richards, T. A., & Talbot, N. J. (2007). Insights from sequencing fungal and oomycete genomes: what canwe learn about plant disease and the evolution of pathogenicity? *Plant Cell*, *19*, 3318–3326.

Taylor, R. D., Saparno, A., Blackwell, B., Anoop, V., Gleddie, S., Tinker, N. A., et al. (2008). Proteomic analyses of *Fusarium graminearum* grown under mycotoxin-inducing conditions. *Proteomics*, *8*, 2256–2265.

Tian, M. Y., Huitema, E., Da Cunha, L., Torto-Alalibo, T., & Kamoun, S. (2004). A Kazal-like extracellular serine protease inhibitor from *Phytophthora infestans* targets the tomato pathogenesis-related protease P69B. *Journal Biological Chemistry*, *279*, 26370–26377.

Torto, T. A., Li, S., Styer, A., Huitema, E., Testa, A., Gow, N. A., et al. (2003). EST mining and functional expression assays identify extracellular effector proteins from the plant pathogen *Phytophthora*. *Genome Research*, *13*, 1675–1685.

Tyler, B. M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R. H., Aerts, A., et al. (2006). *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science*, *313*, 1261–1266.

Valent, B., & Chumley, F. G. (1991). Molecular genetic analysis of the rice blast fungus *Magnaporthe grisea*. *Annual Review of Phytopathology*, *29*, 443–467.

Washburn, M. P., Wolters, D., & Yates, J. R., III. (2001). Large scale analysis of yeast proteome by multidimensional protein identification technology. *Nature Biotechnology*, *19*(3), 242–247.

Whisson, S. C., Boevink, P. C., Moleleki, L., Avrova, A. O., Morales, J. G., Gilroy, E. M., et al. (2007). A translocation signal for delivery of oomycete effector proteins into host plant cells. *Nature*, *450*, 115–118.

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

Xu, S., Chen, J., Liu, L., Wang, X., Huang, X., & Zhai, Y. (2007). Proteomics associated with virulence differentiation of *Curvularia lunata* in maize in China. *Journal of Integrated Plant Biology*, *49*, 487–496.

Zhou, W., Kolb, F. L., & Riechers, D. E. (2005). Identification of proteins induced or upregulated by Fusarium head blight infection in the spikes of hexaploid wheat (*Triticum aestivum*). *Genome*, *48*, 770–780.

Zhou, W., Eudes, F., & Laroche, A. (2006). Identification of differentially regulated proteins in response to a compatible interaction between the pathogen *Fusarium graminearum* and its host, *Triticum aestivum*. *Proteomics*, *6*, 4599–4609.