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Review

Fungal pathogenicity genes in the age of 'omics'

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SUMMARY

The identification of the fungal genes essential for disease underpins the development of disease control strategies. Improved technologies for gene identification and functional analyses, as well as a plethora of sequenced fungal genomes, have led to the characterization of hundreds of genes, denoted as pathogenicity genes, which are required by fungi to cause disease. We describe recent technologies applied to characterize the fungal genes involved in disease and focus on some genes that are likely to attract continuing research activity.

INTRODUCTION

As fungal pathogens have an enormous impact on plant production worldwide, the strategies they use to infect plants and to cause disease are a topic of great interest. A decade ago, we published a review listing about 80 pathogenicity genes of phytopathogenic fungi (Idnurm and Howlett, 2001). Pathogenicity genes were defined as those necessary for disease development, but not essential for the pathogen to complete its life cycle *in vitro*. This definition was based on the observation that disruption of a pathogenicity gene will result in the reduction or complete loss of disease symptoms; such genes are of considerable interest, as they are potential fungicide targets for disease control. Such targets can be discovered by the analysis of genome sequences and transcriptomes. Earlier this year, 16 genome sequences of fungal phytopathogens were available (Gonzalez-Fernandez *et al*., 2010), and many more sequencing projects are underway (http://fungalgenomes.org/). Candidate pathogenicity genes are now easier to functionally analyse because of improvements in efficiencies of transformation technologies and of gene mutation. Thus, today, hundreds of fungal pathogenicity genes have been identified—too many to tabulate. They are listed in several databases, some of which, for example efungi, are designed to identify gene families involved in pathogenicity, whilst others are dedicated to particular organisms and/or interactions (Table 1). For instance, the PHI database focuses on genes that have been functionally characterized with respect to a role in pathogenicity in plants and/or animals (Winnenburg *et al*., 2008). Indeed, plants and animals have very similar innate immune systems, whereby pathogen-associated molecular patterns (PAMPs) recognize pattern recognition receptors in the host to trigger defence responses. In addition, several fungal genes are essential for disease in both types of organism (Sexton and Howlett, 2006). Some of these genes are described later in this review.

As in our 2001 review (Idnurm and Howlett, 2001), we have broadly categorized pathogenicity genes according to where they appear to act during infection. Mutations to genes involved in any of the steps in the infection pathway can affect pathogenicity. The infection pathway initially involves recognition between the fungus and the plant: some fungi enter the host through wounds or natural openings, whereas others penetrate the epidermis using specialized infection structures, such as appressoria. Once within the host, the fungus may act as a necrotroph (killing host cells), a biotroph (obtaining nutrients from its host without killing cells) or as a hemibiotroph (biotroph and necrotroph at different stages of infection); for some fungi, these strict definitions cannot be applied. We acknowledge that classification on the basis of where genes act in the infection pathway is arbitrary, but it is convenient. Clearly, there are pathogenicity genes with pleiotropic effects—for instance, those involved in signalling—and others where, as yet, the site or timing or the mechanism of their effect is unknown.

Effectors belong to a class of fungal genes involved at various stages of infection; these genes are currently receiving a great deal of attention in the literature (Ellis *et al*., 2009; de Wit *et al*., 2009). They encode small secreted proteins that alter host cell structure and function, facilitate infection and suppress or activate effector-triggered immunity (Hogenhout *et al*., 2009). Such proteins are often cysteine rich and expressed highly *in planta*. Generally, they are polymorphic (or deleted) between individuals, and homologues are absent from related species. Their

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Table 1 Databases that describe pathogenicity-related genes in fungi.

Database	Website	Description	Reference
efungi	http://img.cs.man.ac.uk/efungi/database.html	Comparative genomics of 36 fungi	Hedeler *et al*. (2007)
Fungal genome research	http://fungalgenomes.org/	Lists in-progress and published fungal genomes	
Broad Institute	http://www.broadinstitute.org/science/projects/fungal-genome-initiative/fungal-genome-initiative	Genome sequences of 40 fungi	
JGI fungal genomics portal	http://genome.jgi-psf.org/programs/fungi/index.jsf	Comparative genomics of fungi in specific taxonomic groups	
PHI database	http://www.phi-base.org/help.php	Genes involved in pathogenicity in animals and plants	Winnenburg *et al*. (2008)
Magnaporthe grisea, *Oryza sativa* (MGOS) interaction database	http://www.mgosdb.org/	*Oryza sativa* and *Magnaporthe oryzae*: genomic data with an emphasis on host–pathogen interactions	Soderlund *et al*. (2006)
Filamentous fungal gene expression database	http://bioinfo.townsend.yale.edu/index.jsp	Gene expression data of filamentous fungi	Zhang and Townsend (2010)

existence is usually inferred bioinformatically from genome sequences and transcriptome analyses. Small secreted proteins identified in this manner are often assumed to be effectors, even if a function in disease has not been shown. In addition, they are not only present in pathogens, but have also been found in the mutualistic ectomycorrhizal basidiomycete, *Laccaria bicolor*, where they have been proposed to modulate the interaction with hosts, such as poplar (Martin and Nehls, 2009). It is likely that they are present in other mutualistic fungi, but, as yet, few such fungi have been analysed at a molecular or genomic level. Some effectors are avirulence proteins and have a 'gene-for-gene' relationship with resistance proteins in the host. When a fungal avirulence gene is mutated, hosts with the corresponding resistance gene no longer detect the pathogen; this leads to a compatible interaction. Thus, our definition of 'pathogenicity' genes would not cover those of the avirulence class of effectors. Host-specific proteinaceous toxins that have an 'inverse' gene-for-gene relationship with the host, whereby the interaction leads to compatibility (disease), are also effectors (Oliver and Solomon, 2010). By our definition, such genes would be classified as pathogenicity genes. This review describes some of the more recently discovered pathogenicity genes and focuses on those that we believe will attract continuing research activity.

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF FUNGAL PATHOGENICITY GENES

High-throughput approaches are now being exploited with forward genetics to identify pathogenicity genes. T-DNA tagging, an approach regularly used in the model plant *Arabidopsis thaliana*, has been applied to *Magnaporthe oryzae* (Jeon *et al*., 2007), and the use of the *impala* element, a fungal transposon, has generated insertional mutant banks of *Fusarium oxysporum* f. sp. *lycopersici* (Lopez-Berges *et al*., 2009) and *F. graminearum* (Dufresne *et al*., 2008). Of the more than 500 putative pathogenicity-defective mutants of *M. oryzae* tagged by T-DNA, many had defects in multiple traits, such as conidiation, morphology, germination, growth rates and pigmentation (Jeon *et al*., 2007). Indeed, other studies have also shown that many fungal genes affecting disease progression are involved in general growth and development; pathogenicity is affected because the fungal mutants grow so slowly that plant defence responses are elicited and invasion is stopped before the fungus can release its arsenal of disease determinants.

Often, targeted mutations or deletions of candidate genes that have been identified by bioinformatic and/or transcriptome analyses cannot be achieved as some fungi have low levels of homologous recombination. An increase in efficiency of targeted gene disruption has been attained in *Neurospora crassa* by mutation of the *ku80* gene, which leads to a deficiency in the nonhomologous end-joining pathway (Ninomiya *et al*., 2004). This approach has been exploited to increase the efficiency of gene disruption in fungal pathogens such as *Botrytis cinerea* and *M. oryzae* (Choquer *et al*., 2008; Villalba *et al*., 2008). In addition, for fungi that are still not amenable to gene disruption, gene silencing by RNA interference (RNAi) has been applied (Miyamoto *et al*., 2008; Van de Wouw *et al*., 2009). Another constraint to the functional analysis of candidate pathogenicity genes that are in multigene families is the small number of selectable markers available for fungi. A new technology may be able to overcome this constraint. An inducible FLP site-specific recombination system that enables repeated rounds of gene deletion using a single selectable marker—the hygromycin resistance gene—has been applied to *Ustilago maydis* to delete effector genes (Khrunyk *et al*., 2010).

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FUNGAL PATHOGENICITY GENES

Genes involved in the recognition of the host and in signalling

The recognition of the host by the pathogen triggers numerous signalling cascades, giving rise to the expression of genes involved in disease. Indeed, signalling pathways are extremely important in all facets of fungal growth. Some of these pathways lead to the regulation of genes involved in aspects of pathogenicity, such as the formation of infection or feeding structures, the expression of proteins involved in the protection of the pathogen from the host and the production of molecules used to obtain nutrients from host cells. Cutinase2 is an *M. oryzae* gene involved in surface recognition, germling differentiation, appressorial development, host penetration and full virulence (Skamnioti and Gurr, 2007). This gene has been proposed to be an upstream activator of cyclic AMP/protein kinase A and diacylglycerol protein kinase C signalling pathways that ultimately direct appressorium formation and infectious growth by this fungus. Many of the genes involved in the initial steps of signalling pathways have been well characterized. For example, mitogen-activated protein (MAP) kinases have been investigated in plant pathogenic fungi, including *Stagonospora nodorum* (Solomon *et al*., 2005), *B. cinerea* (Schamber *et al*., 2010), *F. graminearum* (Urban *et al*., 2003) and *Claviceps purpurea* (Mey *et al*., 2002). Mutations in the MAP kinase homologues in these fungi result in a loss of pathogenicity, but also affect other phenotypes, such as *in vitro* growth, melanization and conidiation. Furthermore, some MAP kinases are essential for the maintenance of the symbiotic interaction. For instance, a deletion mutant of stress-activated MAP kinase A, *sakA*, of *Epichloe festucae*, a symbiont, has a pathogenic rather than a mutualistic interaction with its host, perennial ryegrass. Deep RNA sequencing has shown that this mutation is associated with changed expression of a range of genes, consistent with the transition from restrictive to proliferative growth (Eaton *et al*., 2010). The authors noted that many of these transcriptional changes may be a consequence of the breakdown of symbiosis rather than a direct effect of the deletion of *sakA*. However, the use of such a mutant provides valuable insights into fungus–plant interactions.

MAP kinases and other key signalling proteins can activate transcription factors that regulate downstream genes required for disease progression. The role of the transcription factor, StuA, has been investigated in a range of fungi, including *S. nodorum* (IpCho *et al*., 2010), *Aspergillus fumigatus*, which causes invasive aspergillosis of humans (Ohara and Tsuge, 2004), *F. oxysporum* f. sp. *lycopersici* (Sheppard *et al*., 2005) and *Glomerella cingulata* (Tong *et al*., 2007). In *S. nodorum*, the product of this gene regulates central carbon metabolism, the production of the mycotoxin alternariol and the expression of effectors (IpCho *et al*., 2010). Although deletion of *StuA* in *S. nodorum* and *G. cingulata* results in loss of pathogenicity on wheat and apple, respectively, its deletion in *A. fumigatus* and *F. oxysporum* does not affect pathogenicity (IpCho *et al*., 2010; Sheppard *et al*., 2005; Tong *et al*., 2007). This suggests that StuA regulatory pathways are not conserved, and that downstream targets of StuA may vary between fungi. Many downstream targets of such signalling pathways remain unidentified. Chromatin immunoprecipitation-microarray technology (ChIP-chip) analysis can be used to identify such targets. This technique has been exploited in *M. oryzae* to identify genes regulated by MoCRZ1, a transcription factor activated by calcineurin dephosphorylation. Deletion mutants of MoCRZ1 show decreased conidiation, some developmental defects and cannot cause disease when spray inoculated because of their inability to form appressoria (Kim *et al*., 2010). Genes regulated by MoCRZ1 include those involved in calcium signalling, small molecule transport, ion homeostasis, cell wall synthesis and vesicle-mediated secretion. Homologues of some of these are pathogenicity genes in animal pathogenic fungi, and are included in the PHI database. As yet, these downstream targets have not been validated by functional analysis.

In contrast with the pleiotropic effects of the transcription factors described above, mutations of the transcription factor SGE1 of *F. oxysporum* f. sp. *lycopersici* result in a loss of pathogenicity with no other major defects, except for a decrease in the number of microconidia (Michielse *et al*., 2009). The SGE1 mutant did not show detectable expression of several effector proteins essential for disease progression. Homologues have been identified in other phytopathogenic fungi, and therefore it would be interesting to determine whether targeted knockouts lead to similar phenotypes in these fungi.

Genes affecting biosynthesis and the integrity of fungal cell walls

The cell wall is a complex structure of interacting polysaccharides, (glyco)proteins and lipids, and its integrity is important for development, growth and pathogenesis. The appropriate synthesis of cell surface polysaccharides of pathogenic fungi is important for pathogenicity. Indeed, in animal pathogens, the cell wall is a target for the development of antifungal compounds, such as echinocandins, which act via noncompetitive inhibition of the synthesis of 1,3-β-glucans (Morrison, 2006). Chitin is a major component of the cell walls of many fungi, and chitin oligosaccharides released from hyphae during infection can act as PAMPs in innate immunity in both plants and animals (de Jonge and Thomma, 2009). In the tomato wilt fungus, *Cladosporium fulvum*, such oligosaccharides are sequestered by the abundantly expressed protein effector Ecp6, which is essential for pathogenicity on tomato (Bolton *et al*., 2008). Ecp6 contains

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LysM domains that bind glycans, such as chitin oligosaccharides, and this interaction prevents chitin-triggered innate immunity (de Jonge *et al*., 2010). It is not known whether effectors containing such LysM domains play similar roles in other fungus–plant interactions.

The importance of fungal cell walls in plant disease has also been implicated by comparative genomics. Proteomes predicted from genomic sequences of 34 fungi and two oomycetes, including pathogenic and nonpathogenic species, were compared by Soanes *et al*. (2008). Classes of proteins identified with particular motifs specific to ascomycete phytopathogens included the Cas1p-like motif, necessary for O-acetylation of polysaccharides, and the yeast cell wall synthesis protein motif, involved in the synthesis of cell surface polysaccharides. Although the role of such domains is unknown, these cell surface polysaccharides have been suggested to help to hide the fungus from plant defences (Soanes *et al*., 2008). However, functional analyses are required to test this hypothesis.

Genes involved in the production of infection structures

Many fungi differentiate appressoria prior to invasion. The development of such structures involves a large number of genes, and mutations in many of them will arrest infection. Genome-wide transcript profiling has revealed that genes involved in amino acid degradation, lipid metabolism, secondary metabolism and cellular transportation are all differentially upregulated during appressorial formation in *M. oryzae* (Oh *et al*., 2008). Indeed, *M. oryzae* is a model system for the analysis of disease processes, particularly appressorial formation. The initiation of this process is dependent on entry of the cell cycle into S-phase prior to mitosis (Saunders *et al*., 2010). Autophagy, a cellular process that involves the degradation and recycling of a cell's own components, is also involved in the development of appressoria, as well as in conidiation and spore germination. Mutations in genes involved in nonselective macroautophagy, but not in genes involved in selective forms of autophagy, lead to reduced virulence in *M. oryzae* (Talbot and Kershaw, 2009). In *U. maydis*, the mutation of two autophagy genes, *ATG1* and *ATG8*, leads to defects in morphogenesis, as well as pathogenicity, on maize (Nadal and Gold, 2010).

Genes involved in the penetration of the cuticle and cell wall

To penetrate plant cells, many fungi produce enzymes that degrade the cell wall. Comparative genomic analysis has shown that gene families encoding such hydrolytic enzymes are expanded in some pathogenic fungi compared with nonpathogenic fungi (Ma *et al*., 2010; Soanes *et al*., 2008). The functional analysis of such genes is difficult because of redundancy in their function, although, as described above, the development of systems in which a single selectable marker can be used to repeatedly delete different target genes should enable the performance of such experiments. As well as cuticle- or cell wall-degrading enzymes, other proteins are involved in penetrating the surface of the plant. A secreted effector, Pep1, of *U. maydis* is essential for the invasion of maize (Doehlemann *et al*., 2009). Disruption mutants of *Pep1* have normal saprophytic growth and develop infection structures, but their growth is arrested during the penetration of epidermal cells and a strong hypersensitive response is elicited. This gene regulates more than 100 plant genes, many of which are involved in defence responses. The *Pep1* homologue in *U. hordei* behaves similarly in the interaction of *U. hordei* with barley, and can complement the mutation in *U. maydis*. The authors propose that this gene has a conserved function in establishing compatibility. It would be interesting to determine whether Pep1 plays such a role in other biotrophic basidiomycete pathogens.

Genes involved in fungal nutrition

Fungi need to obtain nutrients from the host during invasion and, not surprisingly, pathogenicity genes relating to fungal nutrition have been identified. In many of the organisms examined so far, mutations to genes, such as isocitrate lyase and malate synthase in the glyoxylate pathway, which is involved in fatty acid oxidation, lead to a loss of pathogenicity (for a review, see Dunn *et al*., 2009). Furthermore, mutations to proteins specific to organelles, such as peroxisomes, in which the glyoxylate pathway occurs, lead to a loss of pathogenicity (Kimura *et al*., 2001). Imazaki *et al*. (2010) have shown recently that mutations affecting the *Alternaria alternata* peroxin protein, AaPEX6, essential for peroxisome biogenesis, lead to a loss of pathogenicity. This phenotype is also associated with defects in fatty acid oxidation and the production of the secondary metabolite host-specific AK-toxin. As well as fatty acids, sugars such as sucrose are important in fungal nutrition. A high-affinity sucrose transporter (SRT1) of *U. maydis* enables the uptake of sucrose from the apoplast of maize; this gene is expressed only during infection and, if it is mutated, the resultant isolate is nonpathogenic (Wahl *et al*., 2010). This was an unexpected finding, as it has been assumed previously that glucose, rather than sucrose, is transported into the fungus after cleavage of sucrose catalysed by invertase.

Minerals, such as iron, are essential for fungal growth and development. Siderophores are small iron-chelating peptides used by fungi to acquire iron. In four phytopathogenic fungi (*Cochliobolus heterostrophus*, *C. miyabeanus*, *F. graminearum* and *Alternaria brassicicola*), knockouts of genes, such as nonribosomal peptide synthetases, which are involved in the synthesis

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of these siderophores, can lead to reduced virulence as a result of the inability of the fungus to acquire its iron needs *in planta* (Oide *et al*., 2006). Similar mutants of *A. fumigatus* have reduced virulence in an invasive aspergillosis mouse model (Schrettl *et al*., 2007). Instead of using siderophores, *U. maydis* relies on reductive iron uptake. Mutations to iron acquisition genes—*fer1*, which encodes an iron multicopper oxidase, and *fer2*, which encodes a high-affinity iron permease—reduce the virulence of this fungus (Eichhorn *et al*., 2006). Thus, although both ascomycetes and basidiomycetes have very different systems of iron uptake, mutations in key iron uptake genes lead to a loss of pathogenicity, reflecting the importance of these processes to fungal pathogenicity.

Genes involved in host colonization

Toxins often are major components of the arsenal of virulence determinants by necrotrophic fungi. They can be host specific or nonhost specific, and they kill or disable functions of host cells. A small host-specific protein, ToxA, is present in the dothideomycetes *Pyrenophora tritici* f. sp. *repentis* (Cuiffetti *et al*., 2010) and *S. nodorum* (Friesen *et al*., 2006). The gene encoding ToxA can be considered as a pathogenicity gene, as its mutation or deletion gives rise to isolates unable to cause disease on wheat lines with *Tsn1*, the corresponding host gene. A range of host-specific toxins has now been characterized from *S. nodorum* with each of these interacting with the host genes in an inverse gene-for-gene interaction, whereby the host gene confers susceptibility rather than resistance (Friesen *et al*., 2010). ToxA appears to have been acquired by *P. tritici* f. sp. *repentis* from *S. nodorum* by horizontal gene transfer, and the proposed timing of this transfer corresponded to an increase in reports of disease severity caused by *P. tritici* f. sp. *repentis* (Friesen *et al*., 2006). As yet, genes encoding this proteinaceous toxin have not been found in the genomes of other dothideomycetes.

Other fungal toxins are secondary metabolites, some of which are important in disease. The initial step in the biosynthesis of such molecules often involves nonribosomal peptide synthetases or polyketide synthases. These enzymes generally require activation by an Sfp-type 4′-phosphopantetheinyl transferase (PPT1). Mutants of this gene in *Colletotrichum graminicola* have a greatly reduced content of secondary metabolites, including melanin, form very few appressoria and are hypersensitive to reactive oxygen species. These mutants are unable to penetrate maize, although they can colonize wounded plants, but are defective in conidiation and do not cause necrotic anthracnose symptoms (Horbach *et al*., 2009). The authors concluded that the secondary metabolites lacking in these *PTT1* mutants may be necessary for the necrotrophic lifestyle of the fungus. In addition, as expected, such mutants do not form siderophores, nor do they produce lysine, whose synthesis involves a nonribosomal peptide synthetase. It would be interesting to determine whether mutation of this gene diminishes pathogenicity in fungi that do not require appressoria for infection, or that need particular secondary metabolites as a requirement for infection.

Genes whose role in disease is unknown

High-throughput systems of random tagged mutagenesis have identified large numbers of genes involved in pathogenicity, but many of these genes are hypothetical. Such genes are also implicated by comparative genomic analyses. For instance, comparison of the genomes of three *Fusarium* species revealed the presence of four chromosomes specific to *F. oxysporum* f. sp. *lycopersici*, which were rich in transposons and also in genes with distinct evolutionary profiles that were related to pathogenicity (Ma *et al*., 2010). When such chromosomes were transferred to strains of *F. oxysporum*, they converted a nonpathogenic strain into a pathogen. This is the first demonstration of the transfer of large pieces of DNA between isolates leading to the acquisition of pathogenicity, and the finding highlights the potential for the evolution of pathogenic strains via horizontal gene transfer of large pieces of DNA. Other genes with unknown functions play a crucial role in maize smut disease. Sequencing of the *U. maydis* genome revealed 12 clusters of genes encoding small secreted proteins expressed *in planta*. Deletion of five of these clusters altered the virulence, ranging from a lack of symptoms to hypervirulence on maize (Kamper *et al*., 2006). The screening of some of these mutants, as well as maize mutants with organ-specific defects, has shown organ-restricted tumour formation (Skibbe *et al*., 2010). This is an example of pathogenicity genes that appear to function in a plant organ-specific manner. How such genes interact with host factors to modulate biotrophy and tumour formation can now be addressed.

FUTURE DIRECTIONS AND CONCLUSIONS

The development of new and inexpensive sequencing platforms will lead to the identification of even more sets of candidate genes with essential roles in plant disease. However, more accessible and user-friendly bioinformatic methods are necessary to search and analyse data. This issue has been highlighted by Cairns *et al*. (2010), who described their difficulties in analysing microarray datasets, developed by other researchers of genes, expressed by four fungi during infection of their plant or animal host. The approaches described in this review have focused on genomes and transcriptomes. The elucidation of the proteomes and metabolomes of fungi will lead to more knowledge about the disease process (Tan *et al*., 2009). Proteomic analysis of culture filtrates provides information about and validation of secreted proteins; this approach has been applied to identify

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effectors of *Leptosphaeria maculans*, the blackleg pathogen of oilseed rape (Vincent *et al*., 2009). As effectors are such a hot topic in plant pathology, it is likely that more proteomic studies will be carried out to elucidate post-translational modifications and to determine the expression levels of these proteins.

Currently, there is an abundance of candidate pathogenicity genes, but there is a 'bottleneck' for their functional analysis, as such experiments are time-consuming and difficult to carry out for some fungi. However, genomic approaches are already providing a much more integrated picture of pathogenicity mechanisms, compared with the previous focus on individual genes. The realization that fungal pathogenesis in plants and animals has many parallels is encouraging researchers to think more broadly about the attributes of fungal pathogens. As mentioned earlier, many fungal genes affecting disease progression are involved in growth and development, and there are few genes for which the only effect is on disease. Genes with pleiotropic effects include those initiating signalling pathways; functional analysis of their downstream targets should identify genes with specific roles in pathogenicity. Few genes that play a role in pathogenicity across all fungal pathogens have been identified. This is not surprising as the process of infection varies for each pathogen–host interaction. The genes that appear to have a universal role in pathogenicity are those with pleiotropic effects, such as MAP kinases.

The classification of plant–fungus interactions as pathogenic or symbiotic is indistinct and, indeed, some processes may well overlap (Newton *et al*., 2010). Furthermore, the nomenclature pertaining to pathogen genes that are involved in disease is often confusing. Almost 20 years ago, Shaner *et al*. (1992) discussed the use of terms in plant pathology: 'pathogenic' was defined as 'causing or capable of causing disease', and 'virulence' was defined as 'the quantification of pathogenicity'. At that time, our knowledge of the molecular mechanisms of fungal infections and the tools available to study them was rudimentary. However, as fungi have become more amenable to molecular approaches, genes with potential roles in disease have been functionally analysed by mutation. In most cases, the inoculation of a host plant by the resultant mutant has led to reduced rather than complete loss of disease symptoms. Thus, the distinction between a pathogenicity and virulence gene is blurred; indeed, these terms are used interchangeably by many researchers. In addition, the inclusion of genes that have effects on conidiation, such as those involved in some signalling pathways, is debatable. Fungi with mutations of such genes may not be able to complete their life cycle *in vitro*. Thus, such classifications of genes (virulence or pathogenicity) are probably too simplistic to address the complexities associated with fungal diseases. Nevertheless, regardless of what gene nomenclature is used, the approaches of candidate gene discovery and functional analysis will still be exploited to systematically elucidate the mechanisms of fungal pathogenesis.

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