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# Mining Effector Proteins in Phytopathogenic Fungi

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## 1. Introduction

"Pathogen effector" has been increasingly used in the past decades in the plant-pathogen interactions (Hogenhout et al., 2009).Presently, the definition of pathogen effector commonly adopted the definition given by Sophien Kamoun, that is, effectors are 'molecules that manipulate host cell structure and function, thereby facilitating infection (virulence factors or toxins) and/or triggering defense responses (avirulence factors or elicitors)' (Kamoun, 2006). Plant and their related pathogen have coevolved for many millions of years, which resulted in evolving some resistance genes in plants to prevent or limit pathogen infection, and simultaneously pathogen also evolved some effector proteins to overcome plant defense as well as cause disease. Many plant pathogens secreted effector proteins into host cells to repress plant defense and contribute to pathogen colonization and breach (Birch et al., 2006;Chisholm et al., 2006; Grant et al., 2006; Huang et al., 2006 a, 2006 b; Jones and Dangl, 2006; Kamoun, 2006; O'Connell and Panstruga, 2006).

Oomycetes could cause many destructive plant diseases, like potato late blight that caused the Irish potato famine in the nineteenth century (Tyler, 2007). Oomycete effector proteins could be translocated into host cells with the help of RXLR and dEER motifs in the absence of the pathogen (Dou et al., 2008; Whisson et al., 2007).Oomycetes secreted effector proteins into the infection site. The effector proteins were categorized into two classes based on action site of effectors, and one was extracellular effectors acted in the apoplastic space where they interact with extracellular molecules of hosts. The other was cytoplasmic effectors that acted within the boundary of plant cell wall.

Plant fungal pathogen also secreted effector proteins into host cells where they incompatibly interacted with plants receptors encoded by major resistance genes, which rapidly triggered host defense response (Ellis et al., 2006; Tyler, 2002). Oomycete effectors play dual role in disease and plant defense, fungal effector proteins were no exception. For example, victorin of *Cochliobolus victoriae*, NIP1 of *R.secalis*, and AAL toxin of *Alternaria alternate* played the role in toxin(Lorang et al., 2007; Navarre and Wolpert, 1999; Rohe et al., 1995; Spassieva et al., 2002; van't Slot and Knogge, 2002; Wang et al., 1996, Wolpert et al., 2002), but some studies showed *NIP1* and *ToxA* also interacted with corresponding host-resistance or toxin-sensitivity genes, which resulted in *NIP1* and *ToxA* acted in the same way as the *Avr* genes (Schürch et al., 2004; Stukenbrock and McDonald, 2007). So, it is necessary to clarify fungal effector proteins incompatibly or compatibly interact with host receptors when they were

transported into host cells. In the meanwhile, identification and functional assay of fungal effectors-encoding genes will contribute to discovering mechanism for interaction and coevolution of pathogens and plants. In this chapter, we will focus on our recent years' studies on mining, sequence characterization and functional analysis of secreted effector proteins in fungi and model plant of *Arabidopsis thaliana*.

## 2. Mining effector-encoding genes in *Magnaporthe grisea* genome database

*Magnaporthe grisea* is an ascomycete fungus and the causal agent of rice blast disease, which is the most destructive disease of rice-growing areas in the worldwide. The annual rice yield loss caused by blast disease is enough to feed about 60 million people (Ou, 1985).Whole-genome sequence indicated fungal and oomycete plant pathogen had large amounts of secreted proteins (Dean et al., 2005; Kämper et al., 2006; Tyler et al., 2006).*Magnaporthe grisea* genome sequence was available online, which facilitated to mining many novel effector-encoding genes. And some online software could be used to predict some features such as secretion, domain and homology of effector protein. This provided some evidence for next functional verification.

### 2.1 Predicting classically and non-classically secreted effector proteins in *M. grisea*

Secreted effector proteins are secreted from pathogen cell into extracelluar space. Secreted proteins were categorized into two classes based on their secreted pathway, one was classically secreted proteins, there was a signal peptide in N-terminal of proteins, and the other was non-classically secreted proteins, their secreted pathway was known as leaderless secretion (Nickel, 2003).

Classically secreted proteins in *M. grisea* were predicted through combined online software such as SignalP v3.0, TargetP v1.01, big-PI predictor and TMHMM v2.0 (http://www.cbs.dtu.dk/ services/), the determinant standard of classically secreted proteins conformed to the following four standards, the first standard is L=-918.235-123.455×(Mean S score) +1983.44×(HMM score) and L>0 for predicting proteins with N-terminal signal peptide, the second one is proteins with signal peptides were transported via Sec pathway, the third one is no transmembrane, the fourth one is no GPI-anchor site (Samuel et al., 2003).

Total of 12,595 putative proteins including 1,486 small proteins from *M. grisea* database were predicted. Of which, 1,134 putative proteins were predicted for classically secreted proteins with N-terminal signal peptide. Their signal peptide length lied in between 15-45 amino acids. Here, we will center on small secreted proteins (amino acid length <100), there were 119 classically secreted proteins among 1,486 small proteins, we selected 45 putative secreted proteins-encoding genes among 119 genes as candidates in order to analysis their polymorphism in blast strains from Yunnan, China, the results showed that the most of genes distributed in 21 tested blast strains from Yunnan, which indicated high polymorphism and conservative in blast fungus strains. In addition to classically secreted proteins, non-classically secreted proteins were in further predicted using SecretomeP 2.0 Server (http://www.cbs.dtu.dk/services/).

### 2.2 Predicted features of secreted protein sequence

To conveniently identify function of predicted secreted effector proteins, sequence features of secreted effector proteins needed to be predicted. The gene sequence prediction began

with the identification of regions of DNA that coded for expression of proteins. Whether there was intron or not in DNA sequence for eukaryotic genome. Molecular weight of immature protein was important for gene cloning and functional identification. For secreted proteins, subcellular location was needed to be predicted, which contribute to understand organelle in which secreted effector protein interacted with host receptors. In addition, prediction of protein domain was necessary to experimentally assay function of protein in future. For example, it was predicted that many effectors from plant pathogenic *Phytophthora* species had N-terminal motifs (RXLR-dEER) that were necessary to translocate these effectors into host cells (Jiang et al., 2008), along with the motif prediction, many experiments such as oomycete effectors were translocated into host cells and their function had been carried out, So, to some extent, domain or motif prediction experimentally facilitated function identification of effector proteins.

We predicted subcellular location and domain of the parts of secreted proteins. For example, MultiLoc/TargetLoc (http://www-bs.informatik.uni-tuebingen.de/Services/MultiLoc/)was used to predict subcellular location of 8 secreted proteins. The result showed that subcellular location of MGS4 was predicted to be cytoplasmic, and MGS7, MGS11, MGS42, MGS53, MGS60 and MGS174 were predicted to be extracellularly secreted. Theoretical pI of 7 secreted proteins showed that they were predicted to lie in between 7.69-8.13 except MGS4 was 5.71, and their theoretical molecular weight lied in between 8.50-10.70. Their domain prediction revealed MGS7 possessed a transmembrane region, and MGS53 had a domain of ZnF-$C_2H_2$, while other 5 proteins had no any typical domain except a signal peptide sequence contained in N-terminal of proteins. The 8 secreted proteins sequences were searched using BLASTN or BLASTX of NCBI nr database. No high similarity to sequences from other organism was found, which revealed the effector-encoding genes were novel. Collectively, we analyzed molecular weight, subcellular location, domain and homology of putative secreted proteins, which provide a base for their functional prediction and identification.

### 2.3 Analysis of a Host-Targeting Motif and its flanking sequence in the genome of *Magnaporthe grisea*

It is well-known that bacterial pathogens delivered effectors inside plant cells through the type III secretion system (TTSS). The motif is primarily found in the pathogenic protein of *Plasmodium falciparum*, termed RxLxE/D/Q, and its role is to target the host during the export of virulence proteins; this motif is conserved in *P. falciparum*(Marti et al., 2004), and is defined as host-targeting signal (HTS) or host-targeting motif (HTM). In *P. falciparum* genome, this motif is detected within the 60 amino acids downstream of the secretion signal sequence cleavage sites in approximately more than 400 proteins (Hiller et al., 2004). Subsequently, a series of findings suggested that effectors from oomycete such as *Hyaloperonospora parasotica, Phytophthora infestans, Phytophthora sojae,* and *Phytophthora ramorum* possessed the conserved motif, termed RxLR, located within the N-terminal 60 amino acids downstream of signal peptide cleavage sites, which is similar in sequence and position to the Plasmodium sp HTM, also the RxLR motif and HTM domains are functionally interchangeable (Haldar et al., 2006; Bhattacharjee et al., 2006). In summary, these findings indicated that these oomycetes shared conserved machinery for the transport of effectors. Whether did the motif of RxLx exist in effectors from *Magnaporthe grisea*? So, we predicted that the secretory proteins of *M. grisea* possessed the motif RxLx.

Here, we applied a tool of MEME (http://www.meme.sdsc.edu/) to analysis RxLx of 1,270 putative secretory proteins from the fifth edition of the rice blast fungus genome

(http://www.broad.mit.edu/ annotation/fungi/magnaporthe). The results showed that 297 putative secreted proteins possessed the motif of RxLx, the motif located within the region of 100 amino acids downstream of the N-terminal signal sequence cleavage sites. The number of secretory proteins with RxLx motif was similar with those of Plasmodium and Oomycetes Host-targeted secretome, which indicated that the RxLx motif possibly function as transporting of secreted proteins of *M. grisea* into host cells. However, biological experiments are required for further verification.

Weblogo (http://weblogo.berkeley.edu/cache/fileDo8NeU.png) was used to analyze a sequence logo of the MEME motif and its surrounding region of 149 putative secretory protein sequences (Figure1). Arg(R) in position 1 and Leu (L) in position 3 were the most highly conserved residues in the motif RxLx. It also showed the lower but possible finite positional value, the other residues represented as 'x' in the linearized motif RxLx. By contrast, the E/D/Q residues in the 5 amino acids core of the Plasmodium HTM and the enrichment in E/D residues downstream and the highly conservation Arg(R) in position 4 in the motif RxLR that were required for function of motif RxLR were no positionally conserved in secretory proteins containing motif RxLx of *M.grisea*.

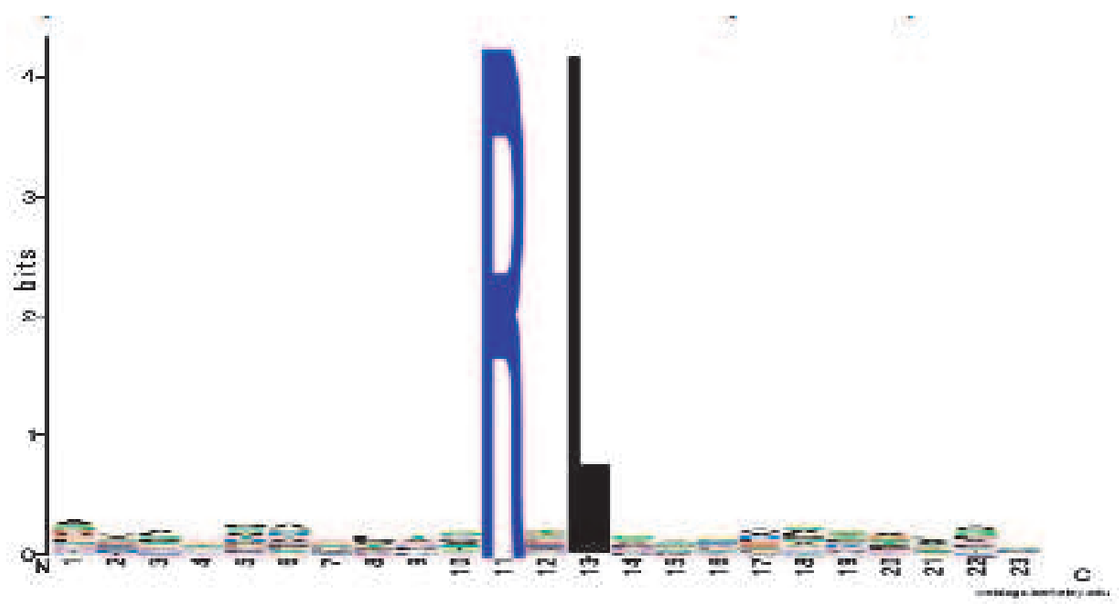



Fig. 1. Sequence logo derived from 149 predicted secreted proteins of *M. grisea*

### 2.4 Functional prediction and analysis of RxLx motif-containing secretory proteins of M. grisea genome

To analyze whether the RxLx motif-containing secretory proteins of *M. grisea* was involved in pathogenicity, we compared the predicted proteins with the PEDANT database. By manually comparing, there were putative ascribed functions of 62 RxLx motif-containing secretory proteins of *M. grisea* (Table 1).These proteins had putative functions such as proteins of *MGG_11036*, *MGG_09159*, *MGG_09248*, *MGG_08401*, *MGG_09143*, *MGG_08424*, *MGG_08537*, *MGG_07809*,*MGG_05479*, *MGG_06008* and *MGG_09460* were possibly related to cell wall degrading enzyme, proteins of *MGG_00922*, *MGG_09817*, *MGG_08436*,

| Gene code | Description |
|---|---|
| MGG_00276 | 6-hydroxy-D-nicotine oxidase |
| MGG_00505 | sun family protein |
| MGG_00671 | arginase family protein |
| MGG_00922 | aspartic proteinase |
| MGG_01085 | conserved hypothetical protein |
| MGG_01195 | conserved hypothetical protein |
| MGG_02853 | nuclease S1 precursor |
| MGG_03029 | neutral proteinase II |
| MGG_03276 | Major allergen Asp f 2 precursor (Asp f II). |
| MGG_03476 | protein-L-isoaspartate(D-aspartate) O-methyltransferase |
| MGG_03670 | vacuolar subtilisin-like serine proteinase SPM1 |
| MGG_03772 | Bile-salt-activated lipase precursor |
| MGG_03995 | carboxypeptidase S1 |
| MGG_04825 | probable endopolyphosphatase precursor |
| MGG_05164 | probable membrane protein YML128c |
| MGG_05479 | xylosidase : arabinofuranosidase |
| MGG_05529 | Feruloyl esterase B precursor |
| MGG_05533 | endochitinase class V precursor |
| MGG_05663 | serine-type carboxypeptidase homolog precursor |
| MGG_05753 | protein disulfide-isomerase precursor |
| MGG_05914 | putative tyrosinase |
| MGG_06009 | alpha-L-arabinofuranosidase |
| MGG_06303 | epsilon-lactone hydrolase |
| MGG_06442 | CATB protein |
| MGG_06538 | blastomyces yeast phase-specific protein 1 |
| MGG_07179 | pepsin C precursor |
| MGG_07234 | FK506-binding protein precursor (Peptidyl-prolyl *cis*- trans isomerase) |
| MGG_07331 | probable GEL1 protein |
| MGG_07502 | SCJ1 protein |
| MGG_07621 | regulator of purine biosynthesis (adenine-mediated repression) |
| MGG_07809 | cellulose 1,4-beta-cellobiosidase |
| MGG_08164 | probable protein disulfide-isomerase precursor |
| MGG_08401 | endoxylanase 11C |
| MGG_08795 | hypothetical protein T10B9.2 |
| MGG_09159 | chitin deacetylase |
| MGG_09162 | L-lactate dehydrogenase precursor |
| MGG_09351 | aspartyl protease |
| MGG_11613 | hypothetical protein B3E4.290 |
| *MGG_12799* | preproalkaline protease |

Table 1. In Silico annotation RxLx-containing secretary proteins of *M. Grisea*

*MGG_03670* and *MGG_03029* were related to proteinase activity, MGG_06662, *MGG_00276*, *MGG_08528*, *MGG_10805*, *MGG_11286* and *MGG_10710* were related to oxidoreductase activity, proteins of *MGG_00238*, *MGG_10219* and *MGG_14395* were associated to reverse transcriptase, proteins of *MGG_08164*, *MGG_05753*, *MGG_02097* and *MGG_11485* were associated to post-translation modification and *MGG_09848* was related to energy activity, which suggested that seretory proteins with RxLx motif in *M.grisea* had diverse functions. Interestingly, among them, some proteins involved in multiple cellular activity, such as *MGG_08164* encoded disulfide isomerase-like protein that involved in cell rescue, defense, energy, development, cell fate and protein folding, modification, destination. In addition, endoxylanse, chitin binding protein, xylanase, pheromone precursor encoded by *MGG_08401*, *MGG_09159*, *MGG_09248*, *MGG_08424*, *MGG_07733*, respectively had been reported that they involved in the pathogenicity of the rice blast fungus. Cellobiose dehydrogenase, cutin hydrolase, endoglucanase, lipase, cellulose encoded by *MGG_11036*, *MGG_01943*, *MGG_08537*, *MGG_09839* and *MGG_07809*, respectively had previously been shown to involve in pathogenicity of other plant fungi (Tudzynski and Sharon, 2003; Mendgen et al, 1996).

## 3. Mining effector-encoding genes in other fungi genome database

Similarly, other fungi genome sequencing had been completed, many fungal genome sequences such as *Fusarium graminearum, Neurospora crassa, Saccharomyces cerevisiae* and *Ustilago maydis* were available online. We analyzed 10,082 proteins from *Neurospora crassa* genome database (http://www.broad.mit.edu/ftp/pub/annotation/neurospora/assembly3 /neurospora_3_protein.gz). There were 437 proteins with signal peptide among total of 10,082 proteins through combined software such as SignalP, TMHMM, TargetP and big-PI Predictior, their signal peptide length lied in between 15 and 59 amino acids. There were 205 predicted secreted proteins that had functional description among 437 proteins, their function mainly involved in diverse enzyme, cell energy, transition, cell recovered and defense mechanism. There were 284 secretory proteins through using software to predict 6,522 protein sequences of *U.maydis* of 284 proteins, 90 proteins contained functional description, and the minimum and maximum of open reading frame were 324 bp and 13,347 bp, respectively. The length range of signal peptides ranged from 16-42 amino acids and the average length was 23 amino acids. Among 284 secreted proteins, 56 proteins possessed the motif of RxLx that located within the region of 100 amino acids downstream of the N-terminal signal sequence cleavage sites.

Similarly, secreted proteins of *Saccharomyces cerevisia* were predicted. N-terminal amino acid sequences of 6,700 proteins of *S. cerevisiae* were available on http://supfam.mrc-lmb.cam.ac.uk/SUPERFAMILY/cgi-bin/gen_list.cgi?genome=sc. *Candida albicans* secretome data were from http://info.med.yale.edu/intmed/infdis/candida/Copyright (2003 John Wiley&Sons, Ltd.). Through the internet-based software such as SignalP v3.0, TargetP v1.01, Big-PI predictor and TMHMM v2.0 prediction for "typical" secretory proteins of *S. cerevisiae*, the 163 secretory ones among the 6 700 proteins were obtained. One hundred and sixty-three predicted secretory proteins were regarded as "Sec-type" signal peptides based on characteristics of four types signal peptides. C-domain of signal peptides of 163 secretory proteins was recognized and cleaved by type I SPases, and had common A-X-A motif, X stood for any amino acid residue. The length and types of amino acid residues of signal peptides were compared between 163 secretory proteins of *S. cerevisiae* and 283 ones of *C.*

*albicans* genome (Table 2 and Figure 2). The result showed that leucine, alanine, serine and valine were found, percentage of leucine was equally 18% in signal peptides of both the two eukaryotic secretomes, but percentage of alanine was 14% in signal peptides of *S. cerevisiae* secretory proteins and 11% in signal peptides of *C. albicans* secretory proteins. Signal peptides composed of a stretch of 19~21 residues in secretory proteins of both *S. cerevisiae* and *C. albicans*. 19 residues composed of signal peptide in *C albicans* secretory proteins had the highest frequency (16.8%), while 20 residues in *S. cerevisiae* secretory proteins had the highest frequency (19.0%).

| Amino acid | Amount and frequency of single amino acid among signal peptide sequences | | | |
|---|---|---|---|---|
| | in *S.* | *cerevisiae* genome | in *C.* | *albicans* genome |
| A | 805 | 14% | 1233 | 11% |
| C | 152 | 2% | 167 | 1% |
| D | 28 | <1% | 79 | <1% |
| E | 46 | <1% | 78 | <1% |
| F | 491 | 7% | 844 | 8% |
| G | 194 | 3% | 385 | 3% |
| H | 77 | <1% | 111 | <1% |
| I | 467 | 7% | 1063 | 9% |
| K | 201 | 3% | 375 | 3% |
| L | 1091 | 18% | 2000 | 18% |
| M | 350 | 6% | 603 | 5% |
| N | 126 | 2% | 251 | 2% |
| P | 94 | 1% | 289 | 3% |
| Q | 127 | 2% | 235 | 2% |
| R | 152 | 2% | 231 | 2% |
| S | 598 | 10% | 1149 | 10% |
| T | 420 | 7% | 847 | 8% |
| V | 978 | 8% | 819 | 7% |
| W | 81 | 1% | 188 | 2% |
| Y | 120 | 2% | 253 | 2% |

Table 2. Single amino acid frequency among predicted signal peptide sequence in *S. cerevisiae* genome and *C. albicans* genome

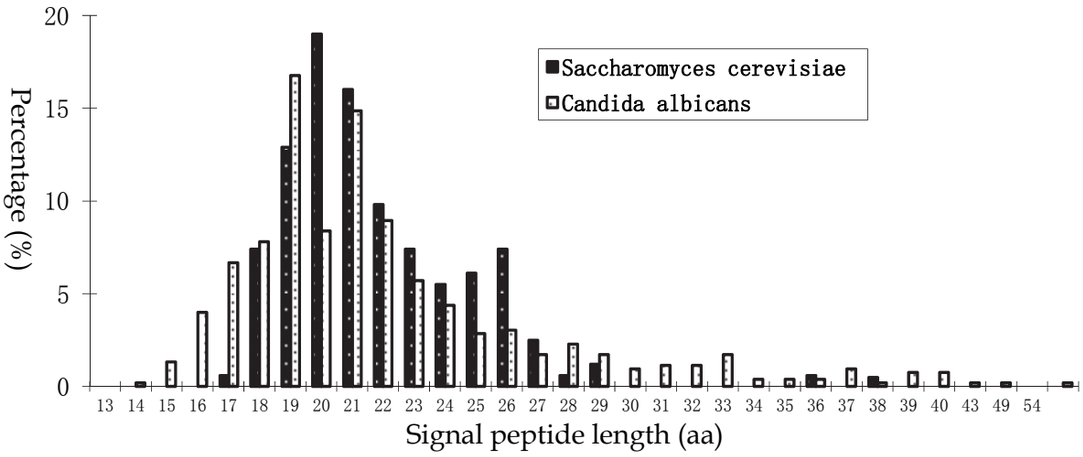



Fig. 2. Length distribution of predicted signal peptides in *S. cerevisiae* and *C. albicans*r

## 4. Mining effector-encoding genes in *Arabidopsis thaliana* genome database

*Arabidopsis thaliana* is the first plant genome that has been sequenced completely. At present, secretome of *Arabidopsis thaliana* and their gene function categorization have not been systematically reported. Here elementally report the prediction of secretome of *Arabidopsis thaliana* and function categorization of gene encoding predicted secretory protein. Combined the computer-based software such as SignalP v3.0, TargetP v1.01, big-PI predictor and TMHMM v2.0 was used to predict the secretome of *Arabidopsis thaliana*. The result showed that 282 secretory ones among 28,953 proteins were obtained, the proportion of predicted secretory proteins to total proteins in entire genome was 2.86 % (Table3). And sublocalization of 282 secretory proteins was further predicted through SubLoc v1.0, their sublocalization was cytoplasmic, extracellular, mitochondrial and nuclear, most of them were secreted into extracellular space, while a few of them were secreted into the other three sublocalization (Table 4).

Based on gene function category (http://webclu.bio.wzw.tum.de/genre/proj/uwe25/Search/Catalogs/searchCatFun.html?id=01), we categorized function of the putative secreted proteins from *Arabidopsis thaliana*. The result showed the most of gene encoding secreted proteins participated in cell metabolism, cell rescue and defense, cell transport, cell fate and storage protein. Among the genes participated in cell rescue and defense, the ratio of genes with functions of peroxidase, kinase, disease resistance protein, pathogenesis-related protein and leucine-rich repeat protein was 50.79%.Among the genes participated in cell metabolism, the ratio of genes with functions of hydrolase, lipase, carboxypeptidase, invertase, transferase, expansin and synthase was 70.33%. It was noteworthy that 724 secretary proteins (18.8 %) were found among 3,848 plant-specific proteins in *Arabidopsis thaliana* genome. Among 2,800 Arabidopsis genes that were reproducibly regulated in response to bacterial pathogen inoculation, 132 genes were potential secretary proteins. These results implied that many plant specific biochemical processes, including pathogen responsive genes were carried out at extracellular space. Prediction of *Arabidopsis thaliana* secretome by the aid of the related computer-based software will accelerate to the experimentally functional study of secretome.

| No. of chromosome | Prediction of SignalP v3. 0 | Prediction of TMHMM v2.0 | Prediction of TargetP v1.01 | | | | Prediction of big-PI predictor |
|---|---|---|---|---|---|---|---|
| | | | S | M | C | - | |
| Ⅰ | 553 | 274 | 202 | 23 | 35 | 14 | 196 |
| Ⅱ | 513 | 248 | 185 | 27 | 23 | 13 | 174 |
| Ⅲ | 633 | 274 | 55 | 29 | 42 | 148 | 55 |
| Ⅳ | 497 | 234 | 191 | 11 | 23 | 9 | 188 |
| Ⅴ | 711 | 307 | 228 | 17 | 41 | 21 | 215 |
| Total | 2907 | 1337 | 861 | 107 | 164 | 205 | 828 |

Note：“S” mean protein with secretory pathway；“M” indicated mitochondrial targeting protein; “C” indicated a chloroplast transit protein; “-” indicated any other location

Table 3. Prediction result through the computer-based software, the SignalP v3.0, TargetP v1.01, big-PI predictor and TMHMM v2.0

| No. of chromosome | Amount of ORF encoding secretory proteins | Amount of total ORFs encoding proteins | Percentage（%） |
|---|---|---|---|
| Ⅰ | 196 | 7494 | 2.62 |
| Ⅱ | 174 | 4589 | 3.79 |
| Ⅲ | 55 | 5742 | 0.96 |
| Ⅳ | 188 | 4407 | 4.27 |
| Ⅴ | 215 | 6721 | 3.20 |
| Summary | 828 | 28953 | 2.86 |

Table 4. Chromosomal distribution of secretory protein in *A. thaliana*

## 5. Expression pattern of effector protein-encoding genes from *M. grisea*

Many studies have used quantitative polymerase chain reaction (PCR) to evaluate fungal growth during the infection process (Hu et al., 1993; Mahuku et al., 1995; Groppe and Boller, 1997; Judelson and Tooley, 2000).Therefore, we detected the expression pattern of candidate novel genes *MGNIP10*, *MGNIP18*, *MGNIP24*, *MGNIP34*, *MGNIP38*, *MGNIP53*, *MGNIP74*, *MGNIP97* and *MgNIP04* in different isolates from Yunnan, China, the same isolate grown under nitrogen-starvation medium and complete medium and different time points when Lijiangxintuanheigu challenged with blast fungus using real-time fluorescence quantitative PCR.

All expression level of candidate genes were normalized by *actin* housekeeping gene and quantified by both the comparative threshold method and standard curve method. The results showed that expression level of all candidate genes were significantly different in

isolates of 94-64-1b Y99-63, 95-23-4a, Y98-16 and 94-64-1b. When two isolates of Y98-16 and Y99-63 grown under complete medium and nitrogen-starvation medium, relative expression quantity of genes was different. And expression of more genes was detected when two isolates grew under nitrogen starvation for 24 h, comparing with when the two isolates grew under complete medium (Figure 3).

We detected expression level of all candidate genes at 24 hpi, 48 hpi, 72 hpi, 96 hpi and 168 hpi, the result revealed that all genes expression level apparently up-regulated, and the expression level achieved the maximum at 48hpi, the expression level had been decreasing after 72hpi (Table 5 and Figure 4).

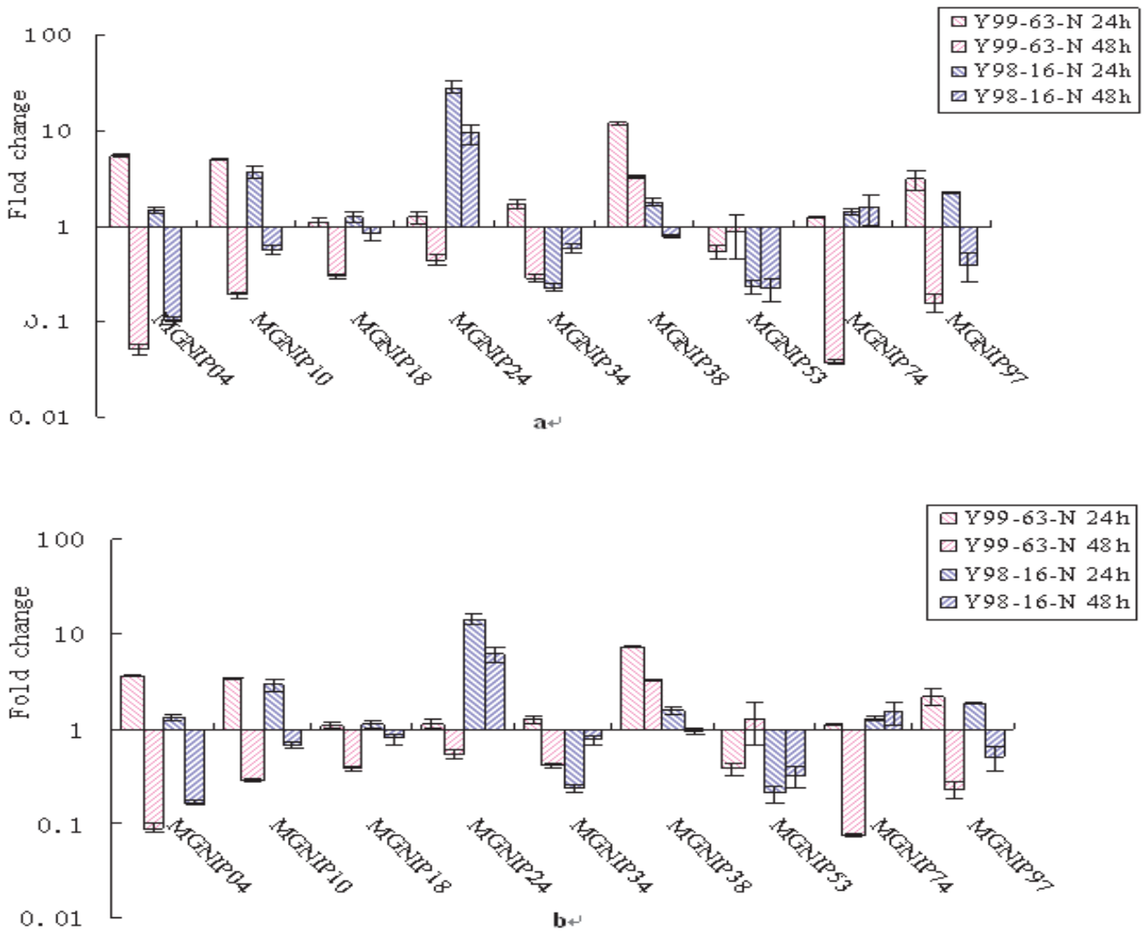


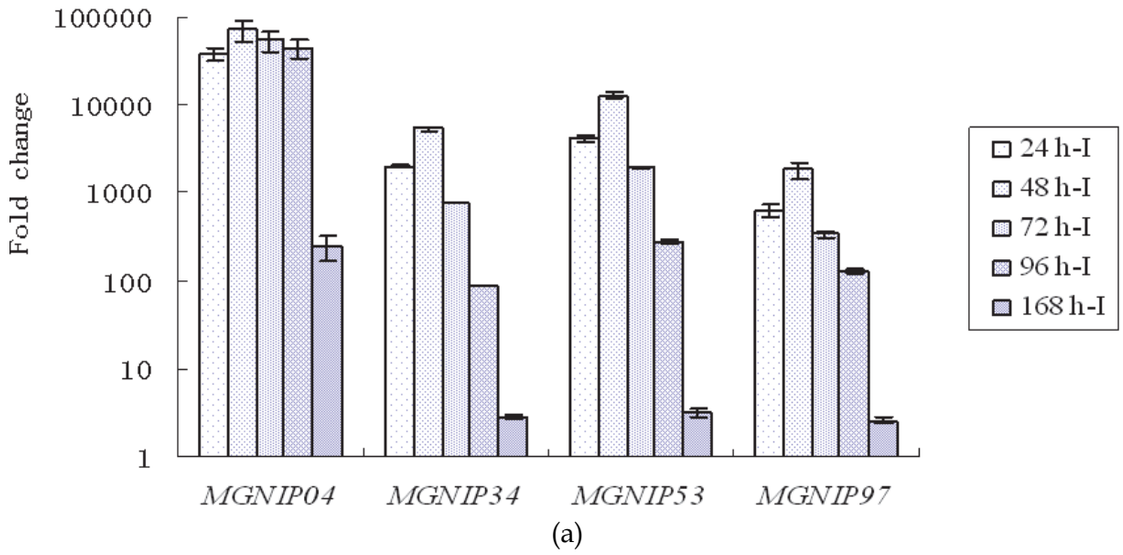

Fig. 3. Expression pattern of some predicted effector protein-encoding genes from *M. Grisea*

a: Relative expression quantity of target gene of Y99-63 and Y98-16 cultured in different mediums by $2^{-\Delta\Delta Ct}$ method

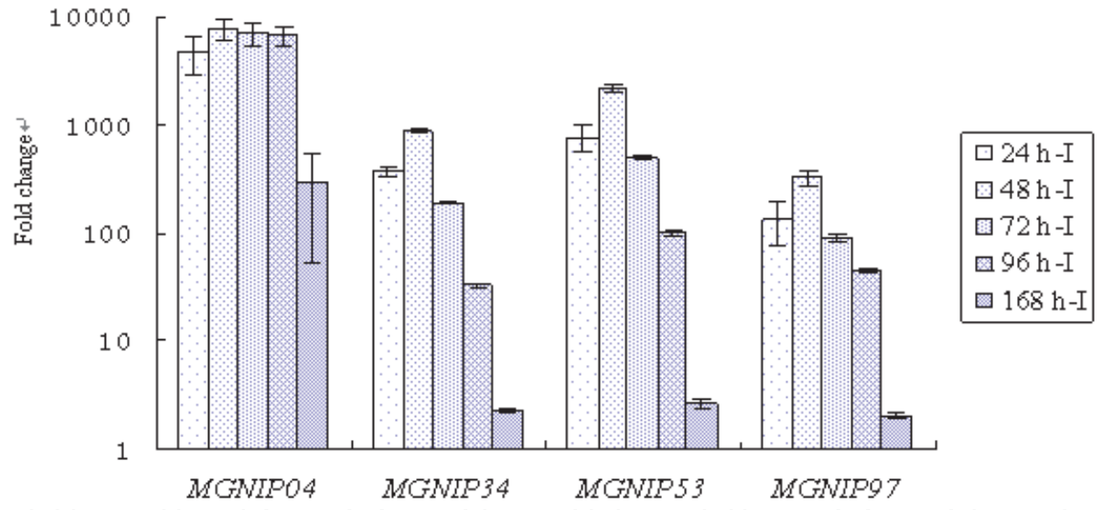
b: Relative expression quantity of target gene of Y99-63 and Y98-16 cultured in different mediums by standard curve method

| Targeted gene \ Isolates | Control | Y99-63 | 24 h-I | | 48 h-I | | 72 h-I | | 96 h-I | | 168 h-I | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $2^{-\triangle\triangle Ct}$ Fold change | Standard curve Fold change | $2^{-\triangle\triangle Ct}$ Fold change | Standard curve Fold change | $2^{-\triangle\triangle Ct}$ Fold change | Standard curve Fold change | $2^{-\triangle\triangle Ct}$ Fold change | Standard curve Fold change | $2^{-\triangle\triangle Ct}$ Fold change | Standard curve Fold change |
| *MGNIP04* | 1 | 1 | 37602±6231 | 4800±854 | 71139±19038 | 7660±1802 | 54388±14248 | 7101±1626 | 44878±10524 | 6812±1360 | 243±76.8 | 291±237 |
| *MGNIP10* | 1 | 1 | 4.88±0.22 | 2.24±0.08 | 8.92±1.04 | 3.63±0.37 | 0.92±0.04 | 0.6±0.02 | 0.29±0.01 | 0.25±0.00 | 0.1±0.01 | 0.13±0.02 |
| *MGNIP18* | 1 | 1 | 1799±201 | 334±28.1 | 2386±743 | 414±95.7 | 359±36.3 | 95±7.37 | 76±4.36 | 29±1.26 | 5±0.48 | 3±0.24 |
| *MGNIP24* | 1 | 1 | 236±37.5 | 77±15.49 | 287±14.8 | 77±3.26 | 68±9.84 | 25±2.95 | 19±0.89 | 10±0.36 | 1±0.06 | 0.6±0.04 |
| *MGNIP34* | 1 | 1 | 2012±68.3 | 376±11.7 | 5401±297 | 879±44.2 | 773±8.74 | 192±1.99 | 88±1.51 | 32±0.51 | 3±0.11 | 2±0.08 |
| *MGNIP38* | 1 | 1 | 1323±121 | 263±21.4 | 1959±512 | 354±79.7 | 282±20.7 | 78±4.98 | 100±17.0 | 36±5.35 | 8±0.57 | 5±0.35 |
| *MGNIP53* | 1 | 1 | 4117±306 | 776±56 | 12888±1242 | 2198±209 | 1931±87.3 | 501±22.5 | 277±14.2 | 101±5.10 | 3±0.27 | 3±0.22 |
| *MGNIP74* | 1 | 1 | 9±2.00 | 5±0.82 | 17±5.22 | 8±1.87 | 5±0.39 | 3±0.19 | 3±0.17 | 2±0.09 | 0.1±0.01 | 0.1±0.01 |
| *MGNIP97* | 1 | 1 | 634±98.7 | 135±18.5 | 1825±385 | 325±59.7 | 337±34.3 | 89±7.86 | 130±9.00 | 45±2.65 | 3±0.19 | 2±0.13 |

Table 5. Relative expression quantity of target gene in infected rice leaves at different stages post inoculation




(a)




(b)

Fig. 4. Expression pattern of some predicted effector protein-encoding genes in infected rice leaves

a: Relative expression amount of target gene in infected rice leaves at different stages post inoculation by $2^{-\Delta\Delta Ct}$ method

b: Relative expression amount of target gene in infected rice leaves at different stages post inoculation by stand curve method

## 6. Pathogenicity analysis of secreted protein from the rice blast grown under nitrogen-starvation medium

Fungi maintained their cell living and even growth through material reutilization when they were in nutrition-stress environment. Some research showed that expression quantity of

pathogenicity-related genes increased when rice blast strains grew under nitrogen-starvation medium, which enhanced the pathogenicity of blast strains (Talbot et al., 1997).
The two isolates of Y99-63 and Y98-16 were from Yunnnan, China. And virulence test of two isolates of Y98-16 and Y99-63 on rice isogenic lines of IRBL1-24 had been previously performed in our lab, and virulence of Y99-63 was more intensive than Y98-16. To analyze the virulence of extracellularly secreted proteins on rice varieties such as susceptible variety of Lijiangxintuanheigu, resistant variety of Tetep and rice isogenic lines of IRBL1-24, we separated the extracellularly secreted proteins when Y98-16 and Y99-63 grew under nitrogen starvation for 48h, and man-made wounded rice leaves were inoculated with extracellularly secreted proteins. The result showed that necrosis speck occurred around the wounded leaves and wounded stems of rice when secreted proteins were inoculated on leaves or stems for 48h, and speck diameter of leaves or stems treated with secreted proteins was 2 to 4 folds larger than leaves or stems treated with sterilized water.
We compared difference of extracellularly secreted proteins from Y99-63 and Y98-16 growing under nitrogen-starvation medium for 48h using two-dimensional electrophoresis technology. The result showed that more proteins spots were detected from Y99-63 growing under nitrogen-starvation medium than Y98-16. And pI and molecular weight of secreted proteins had an apparent difference between Y99-63 and Y98-16.

## 7. Summary

In this chapter, we have showed how mine the secreted proteins from fungi and plant, and how predicted the some features of secreted proteins such as domain, pI, molecular weight and sequence similarity. And simultaneously, we also introduced some experiments centered on expression pattern of secreted protein-encoding genes, and pathogenicity analysis of secreted proteins from the rice blast strains grown under nitrogen-starvation medium.

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## 9. References

Birch, P.R.J., 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.

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.

Dean, R.A, Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thon, M., Kulkarni, R., Xu, J.R. et al. (2005). The Genome Sequence of the Rice Blast Fungus *Magnaporthe grisea*. Nature 434, 980–986.

Dou, D., Kale, S.D., Wang, X., Jiang, R.H.Y., Bruce, N.A., Arredondo, F.D., Zhang, X. & Tyler, B.M. (2008). RXLR-mediated entry of Phytophthora sojae Effector Avr1b into

Soybean Cells does not Require Pathogen-encoded Machinery. Plant Cell 20, 1930–1947.

Ellis, J., Catanzariti, A.M. & Dodds P. (2006). The Problem of How Fungal and Oomycete Avirulence Proteins Enter Plant Cells. Trends Plant Sci. 11, 61–63.

Grant, S.R., Fisher, E.J., Chang, J.H., Mole, B.M. & Hardham, A.R. (2002). Subterfuge and Manipulation: Type Effector Proteins of Phytopathogenic Bacteria. Annu. *Rev. Microbiol*. 60:425-429

Groppe, K. & Boller, T.(1997). PCR Assay Based on a Microsatellite Containing for Detection and Quantification of Epichoe endophytes in Grass Tissue. Appl Environ Microbiol 63:1543–1550.

Haldar, K., Kamoun, S., Hiller, N.L., Bhattacharje, S. & van Ooij, C. (2006). Common Infection Strategies of Pathogenic Eukaryotes. Nature Reviews Microbilolgy. 4:1-12.

Hiller, N.L., Bhattacharjee, S., van Ooij, C., Liolios, K., Harrison, T., Lopez-Estrano, C. & Haldar, K. (2004).A Host-targeting Signal in Virulence Proteins Reveals a Secretome in Malarial Infection. Science. 306:1934-1937,

Hogenhout, S.A., Van der Hoorn, R.A.L., Terauchi, R. & Kamoun, S. (2009). Emerging Concepts in Effector Biology of Plant-associated Organisms. *Molecular Plant – Microbe Interactions* 22, 115–122.

Hu, X., Nazar, R.N. & Robb, J.(1993). Quantification of Verticillium Biomass in Wilt Disease Development. Physiol Mol Plant Pathol 42:23–36.

Huang, G., Allen, R., Davis, E.L., Baum, T.J. & Hussey, R.S. (2006a). Engineering Broad Root-knot Resistance in Transgenic Plants by RNAi Silencing of a Conserved and Essential Root-knot Nematode Parasitism gene. *Proc. Natl. Acad. Sci.* USA103:14302-14306.

Huang, G., Dong, R., Allen, R., Davis, E.L., Baum, T.J.& Hussey, R.S.(2006b). A Root-knot Nematode Secretory Peptide Functions as a Ligand for a Plant Transcription Factor. Mol.Plant Microbe. Interact.19:463-470.

Jiang, R.H.Y., Tripathy, S., Govers, F. & Tyler, B.M. (2008). RXLR Effector Reservoir in Tow Phytophthora Species is dominated by a Single Rapidly Evolving Superfamily with More Than 700 Members. PNAS.105:4874-4879

Jones, J.D. & Dangl, J.L. (2006). The Plant Immune System. Nature. 444: 323–329.

Judelson, H.S., Tooley, P.W. (2000). Enhanced Polymerase Chain Reaction Methods for Detecting and Quantifying Phytophthora infestans in Plants. Phytopathology 90:1112–1119.

Kamoun, S. (2006). A Catalogue of the Effector Secretome of Plant Pathogenic Oomycetes. Annual Review of Phytopathology. 44: 41–60.

Kämper, J., Kahmann, R., Bolker, M., Ma, L.J., Brefort, T., Saville, B.J., Banuett, F., Kronstad, J.W., Gold, S.E. et al. (2006). Insights from the Genome of the Biotrophic Fungal Plant Pathogen Ustilago maydis. Nature. 444:97–101.

Lorang, J.M., Sweat, T.A. & Wolpert, T.J. (2007). Plant Disease Susceptibility Conferred by a "Resistance" Gene. Proc. Natl. Acad. Sci. U.S.A.104:14861-14866

Mahuku, G.S., Goodwin, P.H. & Hall, R. (1995). A Competitive Polymerase Chain Reaction to Quantify DNA of Leptosphaeria maculans During Blackleg Development in Oilseed Rape. Mol Plant-Microbe Interact 8:761–767.

Marti, M., Good, R.T., Rug, M., Knuepfer, E., & Cowman, A.F. (2004).Targeting Malaria Virulence and Remodeling Proteins to the Host Erythrocyte. Science. 306:1930-1933.

Mendgen, K., Hahn, M. & Deising, H. (1996).Morphogenesis and Mechanisms of Penetration by Plant Pathogenic Fungi. Annu.Rev.Phytopahol. 34:367-368

Navarre, D.A. & Wolpert TJ. (1999). Victorin Induction of an Apoptotic/Senescence-Like Respinse in Oats. Plant Cell.11:237-250

Nickel, W. (2005). Unconventional Secretory Routes: Direct Protein Export across the Plasma Membrane of Mammalian Cells. Traffic 6, 607–614.

O'Connell, R.J. & Panstruga, R. (2006). Tete a tete inside a Plant Cell: Establishing Compatibility between Plants and Biotrophic Fungi and Oomycetes. New Phytol.171:699-718.

Ou, SH. (1985). Rice Disease. Kew, England: Commonwealth Mycological Institute.p97-184

Rohe, M., Gierlich, A., Hermann, H., Hahn, M., Schmidt, B., Rosahl, S.& Knogge, W. (1995). The Race-specific Elicitor, NIP1, from the Barley Pathogen, Rhynchosporium secalis, Determines Avirulence on Host Plants of the Rrs1 Resistance Genotype. EMBO14:4168-4177

Lee, S.A., Wormsley, S., Kamoun, S., Lee, A.F., Joiner, K. & Wong, B. (2003). An Analysis of the *Candida albicans* Genome Database for Soluble Secreted Proteins Using Computer-based Prediction Algorithms. *Yeast*, 20:595-610.

Schürch, S., Linde, C.C., Knogge, W., Jackson, L.F.& McDonald, B.A. (2004). Molecular Population Genetic Analysis Differentiates Two Virulence Mechanism of the Fungal Avirulence Gene NIP1, Mol. Plant-Microbe Interact.17:1114-1125

Spassieva, S.D., Markham, J.E. & Hille, J. (2002). The Plant Disease Resistance Gene Asc-1 Prevents Disruption of Sphingolipid Metabolism during AAL-toxin-induced Programmed Cell Death. Plant J.32:561-572

Stukenbrock, E.H.& McDonald, B.A. (2007). Geographical Variation and Positive Diversifying Selection in the Host Specific Toxin SnToxA. Mol. Plant Pathol. 8:321-323

Talbot NJ，McCafeny HRK, Ma M et a1. (1997). Nitrogen Starvation of the Rice Blast Fungus *Magnaporthe grisea* may Act as an Environmental Cue for Disease Symptom Expression. Physiological and Molecular Plant Pathology.50:179-195

Tudzynski, P. & Sharon, A.(2003).Fungal Pathogenicity Genes. Appl Mycol Biotechnol.3:187-212.

Tyler BM.(2002). Molecular Basis of Recognition between Phytophthora Species and Their Hosts. Annu. Rev. Phytopathol. 40:137–167

Tyler BM. (2007). Phytophthora sojae: root rot pathogen of soybean and model oomycete. Mol. Plant Pathol. 8:1–8.

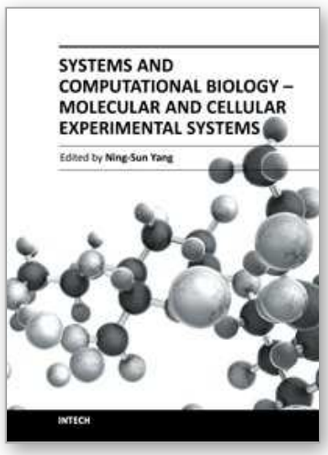
Tyler, B.M., Tripathy, S., Zhang, X.M., Dehal, P., Jiang, R.H.Y., Aerts, A., Arredondo, F.D., Baxter, L., Bensasson, D. et al. (2006). Phytophthora Genome Sequences Uncover Evolutionary Origins and Mechanisms of Pathogenesis. Science 313, 1261–1266.

van't Slot, K.A.E. & Knogge, W. (2002). A Dual Role for Microbial Pathogen-derived Effector Proteins in Plant Disease and Resistance. Crit.Rev. Plant Dis.39:471-482

Wang, H., Li, J., Bostock, R.M. & Gilchrist, D.G. (1996). Apoptosis: A New Model for Perception of Plant Pathogen Effectors. Plant Cell20:2009-2017

Whisson, S.C., Boevink, P.C., Moleleki, L., Avrova, A.O., Morales, J.G., Gilroy, E.M., Armstrong, M.R., Grouffaud, S., van West, P., Chapman. S. et al. (2007). A Translocation Signal for Delivery of Oomycete Effector Proteins into Host Plant Cells. Nature 450, 115–119.

Wolpert, T.J., Dunkle, L.D. & Ciuffetti, L.M.(2002). Host-selective Toxin and Virulence Determinants: What's in a Name? Annu. Rev. Phytopathol.40:251-285

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