Review Article for Fungal Biology Reviews

Title: Delivering the Goods: Fungal Secretion Modulates Virulence During Host-Pathogen Interactions

Short title: Secretion modulates virulence in fungal phytopathogens

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Abstract

Fungi secrete a variety of compounds that have wide ranging beneficial and negative effects on society and govern the outcome of host-pathogen interactions. The secreted compounds range from some of the most powerful toxins and carcinogens, to ethanol used in common commercial practices, and the 'wonder drug' penicillin. Much research in the past 50 years has focused on identifying the genes and their functions relating to the fungal secretome. Recent advances into the mechanisms by which phytopathogenic fungal secretion systems function and modulate virulence

have broad implications for the agricultural and biotechnological industries. In this review, we focus on secretion mechanisms in phytopathogenic fungi with examples from key plant-pathogen systems. Current progress and knowledge gaps regarding secretion pathways and their regulation are discussed. We highlight possible approaches to using novel molecular techniques to generate alternative control methods to synthetic pesticides.

Key words: Mycotoxins, fungi, phytopathogens, secretion, virulence.

I. Introduction

Fungi are ubiquitous organisms that secrete a wide range of compounds, small molecules, and proteins that allow them to externally digest and obtain nutrients from their environments. The process of secretion is conserved throughout the fungal kingdom and plays major roles in survival and proliferation. The molecules secreted by fungi have broad implications for society as they include harmful and potentially deadly toxins. The danger and importance of these mycotoxins gained widespread attention in the 1960's from the Turkey X disease in which aflatoxin, a mycotoxin produced by *Aspergillus flavus*, was responsible for the death of up to 100,000 turkeys (Blount 1961; Nesbitt et al. 1962). However, the impact of mycotoxins dates back much further than the Turkey X mycotoxicosis outbreak. Hypotheses have suggested that the high human death rates of the bubonic plague in the 1300s could have been due to the immunosuppressant effects of mycotoxins in moldy grains (Matossian 1989).

Although many studies have focused on the harmful nature of fungal secretion products, there are also many that have beneficial aspects. Ethanol, secreted by the budding yeast *Saccharomyces cerevisiae*

49 is used to produce beer, and biofuels (Mohd Azhar et al. 2017; Parapouli et al. 2020). Penicillin, the
50 first broad spectrum antibiotic, was originally discovered as a secretion product of *Penicillium rubens*
51 and has been hailed as one of the greatest discoveries in modern medicine (Fleming 1929). Fungi
52 have also been employed as 'cell factories' to produce a diverse range of secondary metabolites that
53 have various industrial applications (Cairns et al. 2019; Meyer et al. 2016). Therefore, understanding
54 the mechanisms and products of fungal secretion has wide-ranging economic and health
55 implications.
56
57 The biological mechanisms behind secretion are complex. Conserved processes within all lineages of
58 fungi, including budding yeast, model filamentous fungi and phytopathogenic fungi are detailed in
59 Figure 1. The secretory machinery consists of a network of molecular entities that are involved with
60 protein folding, transport, maturation and secretion (Delic et al. 2013). These machineries consist
61 mainly of SEC proteins that are essential for membrane fusion, transport vesicles, and molecular
62 switches e.g. GTPases (Schekman 2002). Other integral parts of the secretion system include vacuole
63 protein sorting proteins (VPSP) and the plasma membrane soluble NSF/alpha SNAP receptors
64 (SNAREs) (Wickner and Schekman 2008). In filamentous fungi, the secretory pathway is larger and
65 encompasses more processes than present in yeast (Celińska and Nicaud 2019; Liu et al. 2014; Ohno
66 et al. 2011). For example, filamentous fungi have a larger predicted secretome size [See *Aspergillus*
67 spp. (predicted secretome=757) and *Penicillium* spp. (620.5) vs *Candida* spp. (241.5), and *Saccharomyces*
68 *cerevisiae* (156) from Lum and Min (2011)] and exhibit an increase in the RAB GTPase protein
69 families and SNARE proteins (Swenned and Beckerich 2007).
70
71 The genes that encode the proteins and enzymes affiliated with secretion are often clustered in the
72 fungal genome (Keller 2015) and are regulated by environmental stimuli such as pH, light,

temperature, carbon dioxide, oxygen and nutrients (Alkan et al 2013; Sarikaya-Bayram et al. 2015; Selvig and Alspaugh 2011; Tannous et al. 2020). Homologs of genes involved in secretion exist between model fungi and phytopathogens (Li et al. 2017; Soanes et al. 2008; Wang et al. 2018; Yan et al. 2020). In recent years, research evaluating the genes and molecules in the secretion pathway of filamentous phytopathogens has increased, as novel mechanisms to mine the genome, have shed light on the impact of fungal secretion on virulence during host-pathogen interactions (Jurick II et al. 2019; Levin et al. 2019a; Levin et al. 2019b; Tannous et al. 2018).

Functional genes related to the secretory pathway are essential for virulence, and thus, for the proliferation and success of fungi (Schaller et al 2005; Sorgo et al. 2013). Virulence, (the severity of disease caused by an organism), and pathogenicity (the ability of an organism to cause disease) are complex processes dictated by a variety of host-pathogen interactions. Identifying key components, in the fungal secretome, for functional characterization that are associated with virulence and pathogenicity may yield novel and efficient approaches to control detrimental plant pathogens in the agricultural industry. Deletion of genes associated with the secretion pathway often results in decreased virulence (Jurick II et al. 2019; Levin et al. 2019; Tannous et al. 2018). In the following review we will discuss fungal secretion factors in plant pathogenic fungi with a focus on those that modulate virulence. Additionally, we will emphasize cutting-edge genomic, genetic and fundamental cell biology approaches, tools and concepts concerning secretion and how this work can lead to next generation controls.

II. The Secretome of Phytopathogenic Fungi

96 The molecules secreted by phytopathogenic fungi associated with virulence are versatile and
97 abundant. Examples include mycotoxins used for host colonization (Ismaiel and Papenbrock 2015),
98 and proteinaceous effectors (Selin et al. 2016) involved in host recognition and manipulation (Figure
99 2). Although outside the scope of this review, smaller compounds released from fungi via membrane
100 transport and diffusion also play a role in virulence. These include metabolites such as quorum
101 sensing molecules (e.g. farnesol, phenylethanol tryptophol, and tyrosol), produced by multiple
102 phytopathogens (*Aspergillus* spp., *Penicillium* spp. etc), that enable coordinated gene expression during
103 pathogenesis (Albuquerque and Casadevall 2012; Mehmood et al. 2019), and volatile organic
104 compounds (Hung et al. 2015; Morath et al. 2012).
105
106 Host plants have evolved mechanisms to respond and defend themselves against fungal secretion
107 products. In general, the intercellular interface between the fungal cells and the host is composed of
108 compounds generated by both organisms. From the plant, volatile organic compounds, CWDE (cell
109 wall degrading enzymes)-inhibitors, Polygalacturonase Inhibiting Protein (PGIP), and damage-
110 associated-molecular-patterns (DAMPs) are released into the intercellular space to trigger the plant
111 immune system or to combat the toxins, effectors, and other secondary metabolites exuded by
112 fungi (Kalunke et al. 2015; Xu et al. 2019).
113
114 Fungi within the genera *Aspergillus, Cladosporium*, *Collectotrichum*, *Fusarium* and *Penicillium* produce
115 mycotoxins on agricultural commodities which can be detrimental to the health of the humans and
116 animals that consume them. Some of the most well studied mycotoxins produced by these fungi
117 include aflatoxin, citrinin, and patulin. Aflatoxin, produced by *Aspergillus* species, occurs on multiple
118 grain crops (Jelinek et al. 1989; Kensleer et al. 2011). Outbreaks of aflatoxin are relatively common
119 and can cause acute illness and death in severe cases when the infected crops are ingested (Azziz-

120 Baumgartner et al. 2005; Krishnamachari et al. 1975; Reddy and Raghavender 2007). Citrinin is
121 produced by species in the genera *Aspergillus*, *Monascus* and *Penicillium*, tends to be found on grain
122 crops (Čulig et al. 2017; Föllmann et al. 2014) and has been found to be nephrotoxic (Flais and
123 Peraica 2009; Yu et al. 2006). Patulin, produced by *P. expansum*, is generally associated with
124 postharvest fungal fruit pathogens (McKinley and Carlton 1991) and is commonly found in juice,
125 fruit butters, and cider. In addition to the aforementioned symptoms, both patulin and citrinin are
126 also potential carcinogens (Knasmüller et al. 2004).
127
128 While many studies have highlighted the health implications of mycotoxins in humans, there have
129 also been studies involving their impact on the host plant. Three recent studies evaluated the role of
130 citrinin and patulin on *P. expansum* virulence.. It was found that patulin is a virulence factor as it is
131 involved in host necrosis and fungal colonization (Jurick II et al. 2019; Sanzani et al. 2012; Snini et
132 al. 2016). In other fungal species, such as *Fusarium* spp., production of mycotoxins like
133 deoxynivalenol (DON) can result in stunted growth and reduced plant germination (Masucda et al.
134 2007).
135
136 Besides mycotoxins, major groups of molecules secreted by fungi that modulate virulence are
137 proteinaceous effectors and carbohydrate-active enzymes (CAZymes). Effectors are small molecules
138 that dictate the outcome of the plant-microbe interaction (Selin et al. 2016). The main function of
139 pathogen effectors is to interfere with host plant pathogen-associated-molecular-patterns (PAMP)-
140 triggered immunity and subsequent effector-triggered immunity. The ability of a pathogen to
141 successfully colonize and proliferate on their hosts depends on a variety of effectors secreted by the
142 phytopathogen. Selin et al. (2016) provides a thorough review on some well-studied effectors found
143 in phytopathogenic fungi. Carbohydrate-active enzymes (CAZymes) are a diverse set of proteins

144 secreted by fungi that degrade plant cell wall polysaccharides (Glass et al. 2013; Kubicek et al. 2014).
145 They have been shown to be virulence factors contributing to fungal growth and development in
146 multiple systems by aiding their invasion into host cells (Brito et al. 2006; Kema et al. 2008; Ma et al.
147 2019; Van Vu et al. 2012).
148
149 Research on molecules secreted by phytopathogens is an expanding area of research. Contemporary
150 methodologies, such as comparative genomics, computational biology, transcriptomics, and the
151 CRISPR/ Cas9 system, have and will continue to contribute to the development of next-generation
152 methods to control fungal phytopathogens. For example, organisms can be engineered, and
153 employed as biological control agents, that target and break down some of the molecules within the
154 secretome of phytopathogenic fungi. New bioinformatic techniques are already being implemented
155 such as the effector prediction pipeline developed by Levin et al. (2019) which allows the relatively
156 rapid identification of effectors secreted by phytopathogenic fungi that can then be verified by
157 functional genetic approaches. The rapid identification of effectors will greatly enhance researchers'
158 abilities to locate and study their function. We expect future insights into the phytopathogenic
159 secretome to continue to contribute groundbreaking innovations to the global agriculture sector.
160
161 **III. Fungal Secretion Machineries**
162
163 Phytopathogens contain specific receptors that allow them to recognize their host and initiate
164 pathways that lead to the secretion of hundreds of proteins from intracellular compartments to the
165 exterior of the cell (Jiang et al 2018; Tyler 2002). The secretion of small molecules by fungi is
166 primarily accomplished through the classical and non-classical (bypassing of the golgi) routes. There
167 is also a third route, where small molecules such as mycotoxins are secreted through the formation

of exosomes/toxisomes via cytoskeletal reorganization. The machineries involved with the different secretion pathways of fungal phytopathogens consist of a variety of proteins including SEC proteins, GTPases, vacuole protein sorting proteins (VPSP), and the plasma membrane soluble NSF/alpha SNAP receptors (SNAREs).

In the classical route, proteins are synthesized in the cytosol and then co-translationally translocated to the endoplasmic reticulum where they are glycosylated or decorated with other carbohydrate/lipid moieties. Proteins are transported by vesicles to the Golgi and then to the exterior of the cell and typically require a N terminal signal peptide for translocation (Delic et al. 2013) (Figure 1). Virulence genes associated with the secretion pathway within phytopathogens tend to have homologs in ancestral yeast species such as *Saccharomyces cerevisiae* (Gijzen and Nuernberger 2006; Jurick II et al. 2019; Levin et al. 2019; Nadal et al. 2010). Locating these orthologs in phytopathogenic fungi can fill gaps in the literature on their secretory mechanisms and how they differ from model filamentous fungi and yeasts. Traditionally, both forward and reverse genetics approaches are used to determine the role of the genes involved in phytopathogen secretion and virulence. For example, a reverse genetics approach was used to identify the *S. cerevisiae* ortholog to the CWDE transcription factor, *snf1,* in the fungal corn pathogen, *Ustilago maydis*. The Δ*snf1*strains exhibited reduced virulence when inoculated on maize (Nadal et al. 2010). There are six homologs of the conserved NLP gene family (Gijzen and Nuernberger 2006) that have been identified in the *Colletotrichum higginsianum* genome (Kleemann et al. 2012). Other examples of homologs identified, that are involved with the secretory pathways of phytopathogens, include the *sntB* gene, found in *Aspergillus* (Pfannenstiel et al. 2017), and its ortholog, the *snt2* gene, found in *Fusarium oxyporum* (Denisov et al. 2011a)*, Neurospora crassa* (Denisov et al. 2011b), and *Magnaporthe orzae* (He et al. 2018). The *sntB* gene was recently found to be a virulence factor in *P. expansum* in which mutants exhibited reduced mycotoxin production,

conidiation and virulence (Tannous et al. 2020). *Sec4*, a gene encoding a Rab GTPase in *S. cerevisiae*, that is involved with protein secretion and vesicule trafficking, has a homolog in the corn pathogen *Fusarium verticilliodes*, *FvSec4* (Goud et al. 2008; Salminen and Novick 1987; Yan et al. 2020). Δ*FvSec4* mutants exhibited decreased virulence and decreased production of the mycotoxin fumonisin B1 (Yan et al. 2020). As fumonisin B1 has been associated with virulence in other fungal pathogens these results were not unexpected (Desjardins et al. 1995). Furthermore, *FvSec4,* attached with a green florescent protein, was found in growing hyphal tips leading to the hypothesis that *FvSec4* is associated with protein trafficking in *F. verticilliodes* which is the evolutionary conserved function of *Sec4* in all eukaryotes (Yan et al. 2020). In a different system, the Rab GTPase *CLPT1,* was found to be a pathogenicity factor in the fungal pathogen of bean, *Colletotrichum lindemuthianum* (Siriputthaiwan et al. 2005). *CLPT1* is known to be involved with the transport of vesicles from the Golgi to the plasma membrane and can complement the yeast *Sec*4 mutant (Dumas et al. 2001).

The unconventional pathway usually does not require a signal peptide, as the Golgi is bypassed, and proteins are transported to endosomes/vacuoles following post-translational modification before they are excreted (Rabouille 2017; Miura and Ueda 2018). It should be noted that some proteins secreted via the unconventional pathway require a signal peptide for initial translocation into the endoplasmic reticulum. The unconventional pathways are commonly associated with proteins involved in fungal virulence (Giraldo et al. 2013; Jurick II et al. 2019; Miura and Ueda 2018; Reindl et al. 2019). In some instances, such as with DON produced in *Fusarium graminearum*, the causative agent of *Fusarium* head blight, the mature toxin is developed intracellularly and transported within toxisomes, a specialized endosome vesicle that are proliferations of the smooth endoplasmic reticulum (Boenisch et al. 2017; Menke et al. 2013). The assembly of these toxisomes are provided support by the α1 and β2 tubulins, as such, the disruption of these microtubules disrupts DON

biosynthesis (Zhou et al. 2020). Additionally, myosin1 molecular motors are involved with toxisome formation and mycotoxin production, as inhibition of myosin 1 leads to decreased DON production (Tang et al. 2018). Toxisomes are also thought to play a role in the synthesis of other mycotoxins within *F. graminearum* such as Culmorin (Flynn et al. 2019). For other secondary compounds, e.g. patulin, the last step of the biochemical synthesis (conversion of the non-toxic ascladiol intermediate to the final mycotoxin, patuin via the secreted enzyme Pat E) occurs outside of the fungal cell to separate the fungus from the adverse effects of its own toxin which serves as an auto resistance mechanism (Jurick II et al. 2019; Li et al 2019).

The fungal secretion machineries that are part of the unconventional pathways tend to consist of extracellular vesicles (Rizzo et al. 2020). Extracellular vesicles are secreted membrane vesicles such as exosomes and micro vesicles (van Niel et al. 2018). These vesicles are well known in yeasts and have recently been described in phytopathogens including *Fusarium oxysporum* f. sp. *vasinfectum* where they were hypothesized to be linked to pathogenicity (Bleackley et al. 2019). Additionally, large protein families such as SNAREs play a role in vesicle mediated transportation. In the rice blast fungus, *M. oryzae*, multiple SNAREs have been identified that affect pathogenesis and/or virulence (Dou et al. 2011; Li et al. 2017; Song et al. 2010). Interestingly, in *M. oryzae*, effectors can be secreted either by the conventional pathway (ER-Golgi) or through exocyst components and the Sso1 *t*-SNARE. It should be noted that the conventional pathway is associated with secretion of apoplastic effectors whereas exocyst components, such as EX070 and SEC5, are required for efficient secretion of cytoplastic effectors (Giraldo et al. 2013). In the plant pathogen *F. graminearum,* deleting a gene associated with the *t*-SNARE protein, Sso2*,* involved with secretion, causes decreased pathogen virulence. Single mutants of both Δ*sso2* and, an ATP-binding cassette transporter, Δ*abc,* reduced production of the mycotoxin DON while a double mutant had an additive effect on DON in planta

240 (O'mara et al. 2020). Additionally, in *F. graminearum*, the SNARE's, FgSso1, FgVam7 and FGVps39
241 are known to be important virulence factors (Li et al. 2017). In another phytopathogenic fungus,
242 *Verticillium dahliae* (vascular wilt disease), two SNARE encoding genes, *VdSec22* and *VdSso1* are
243 required for full virulence on cotton plants and were found to be homologous to the yeast SNARE
244 encoding genes *Sec22* and *Sso1* (Wang et al. 2018).
245
246 Furthermore, there have been many studies that have linked gene regulation of the secretion of
247 enzymes and proteins involved in virulence to environmental stimuli (Barad et al. 2015; Hadas et al.
248 2007; Jurick II 2010; Jurick II et al. 2012; Kumar et al. 2017; Prusky et al. 2004; Yao et al. 1996). For
249 example, the effect of pH and *pacC* regulation on the enriched expression of genes associated with
250 host-cell-wall degradation have been reported. Fungal genes with known functions associated with
251 virulence at low pH include chitinase-associated genes, pectin lyase, and polygalacturonase (PG)
252 activities (Jurick II 2010; Jurick II et al. 2012; Yao et al. 1996). Conversely, the over-representation
253 of aspartic endopeptidase-pep1, which is associated with pH modulation of *P. digitatum* and catalyzes
254 hydrolysis of elastin and collagen (the major structural proteins of cell membranes), plays a
255 significant role in the virulence of *P. digitatum* on citrus fruits (Ballester et al. 2019). Aspartic
256 endopeptidase was up-regulated during infection of citrus fruits, and contributed *P. expansum*
257 colonization, either by degradation of plant cell-wall components to provide a nitrogen supply, or by
258 inactivating defense proteins. This type of response at low ambient pH is probably a result of
259 accumulation of gluconic acid (GLA) during the virulence process to ensure that secreted enzymes
260 and metabolites are produced at the optimal pH to facilitate their physiological functions (Barad et
261 al. 2016). Additionally, several reports have indicated different responses of *Penicillium* under
262 different nutritional regulation (Jurick II 2012, Barad et al. 2015). High-sugar content fruits may
263 enhance the acidification of the environment and GLA accumulation, and ammonia produced under

nutritional limitation and low-pH conditions in the host seems to play a central role in the activation of *pacC* responsiveness (Hadas et al. 2007; Prusky et al. 2004). The modulation of gene expression and secretion mechanisms involved with virulence may also be affected by host physiology. Kumar et al. (2017) reported that Δ*laeA* mutants lacking the secretion of patulin, showed 25% reduction in disease severity in mature fruit (high sugar content, 24% TSS) compared to early harvested fruit (low sugar content, 12% TSS) fruit.

Genes in phytopathogenic fungi often have homologs in model systems (Goud et al. 2008; Salminen and Novick 1987; Wang et al. 2018; Yan et al. 2020). The difficult nature of phytopathogens has caused many significant gaps in the literature regarding the function and regulation of all their genes related to the fungal secretion pathway(s). These challenges include their large genomes and obligate parasitic lifestyle, as well as the discrepancies in their gene annotation and characterization. However, functional genomic approaches, using novel gene editing and bioinformatic tools will facilitate major leaps forward in our understanding of biochemical knowledge of the pathways, machinery, and regulators of the fungal phytopathogenic secretome for pathogens with diverse lifestyles that include necrotrophs, hemi-biotrophs, and strict biotrophs.

IV. Capitalizing on secretion to formulate next-generation controls

Secretion of compounds, such as mycotoxins by phytopathogenic fungi have major economic implications and have been estimated to cause upwards of 500 million dollars annually to the United States Agriculture Industry (Figure 3) (Robens and Cardwell 2003). Current mechanisms to control phytopathogenic fungi and their mycotoxins involve intensive fungicide and chemical regimes that can be damaging to the environment. Current control strategies involve the application of intensive,

single-site mode of action, synthetic fungicides. Overreliance on these fungicides can lead to the emergence of resistant fungal populations (Bertrand and Saulie-Carter 1978; Rosenberger 1990; Sholberg and Haag 1996). Cultural and biological control strategies are commonly implemented into integrated pest management (IPM) programs and fungicide resistance management strategies to supplement and limit the use of synthetic pesticides. Unfortunately, their efficacy is often inferior to synthetic fungicides (Moparthi and Bradshaw 2020). Breeding resistant cultivars of agricultural crops is an important part of an IPM plan. However, there has been limited success breeding commercial fruit cultivars (e.g. apples) that are resistant to post-harvest pathogens (Janisiewicz et al. 2008; Jurick II et al. 2011; Luo et al. 2020).

The study and evaluation of how fungal secretion modulates virulence can lead to the development of next-generation controls and innovations for the global agriculture industry. These include the development of antifungals, biological control agents, and bioherbicides. Phytopathogens secrete a range of chemically diverse compounds (Grijseels et al. 2016) that can be toxic to both bacteria and plants (Ismaiel and Papenbrock 2015; Venkatesh and Keller 2019). Effectors and other secretion products have already begun to be evaluated in disease resistance breeding programs against phytopathogens (Vleeshouwers and Oliver 2014).

Manipulating protein expression and secretion in fungi and their host plants through genetic engineering has broad implications for modern agriculture and plant pathological studies. One possible avenue could be to engineer the host plant to include chemistries that interfere with the different pathways and regulators of secretion in phytopathogens. Targeting secretory processes may be advantageous as it could limit the negative effects of fungi (such as mycotoxins) while having minimal impacts on non-target organisms i.e. researchers could target processes that are species or

312 genus specific. Additionally, as many of the molecules secreted by fungi are involved in competition
313 (Keller 2015; Künzler 2018; Venkatesh and Keller 2019), organisms such as yeast or bacteria can be
314 engineered into cell factories that synthesize secretion products of phytopathogenic fungi that have
315 antimicrobial properties. These synthesized compounds can be formulated into commercially
316 available broad spectrum or potentially species-specific fungicides. Using yeast or bacteria as cell
317 factories could be a valuable tool to mass produce, study, and utilize secretion products from hard to
318 cultivate phytopathogens i.e. obligate pathogens such as powdery mildews and rusts. Additionally,
319 the evaluation of the fungal secretome can lead to control methods that can be used in organic
320 agriculture production. For example, non-virulent biocontrol agents can be engineered to
321 outcompete phytopathogens. This tactic is based on previous research that used a naturally
322 occurring, non-toxigenic, *Aspergillus flavus* mutant as a biocontrol agent, Afla-guard™ (Syngenta), to
323 outcompete toxigenic strains of *Aspergillus flavus*.
324
325 Another area of research could be to generate environmentally friendly herbicides. The chemicals
326 that fungi exude are often phytotoxic (Wipfler et al. 2019) and can be host specific (Meena and
327 Samal 2019). These toxins can be analyzed and synthesized as potential organic, environmentally
328 friendly, selective bioherbicides. For example, Zearalenone and DON are toxic on germinating corn
329 embryos and can be analyzed as potential pre-emergent herbicides (Mclean 1995). As for postharvest
330 pathogens, one area that is generally untapped is the molecular aspects that govern decay. Blocking
331 the signaling pathways and regulators of decay is a potential route to the generation of novel crop
332 protection strategies.
333
334 Fungi contain an array of secretion products, particularly secondary metabolites, that have the
335 potential to be used by the agriculture industry as 'organic,' 'environmentally friendly,' pesticides.

The genes associated with these products tend to be arranged in a biosynthetic gene cluster (Keller 2015; Venkatesh and Keller 2019) and by using genomic methods, biosynthetic gene clusters from a range of phytopathogenic fungi can be mined and engineered into cell factories to locate, analyze and synthesize a range of secretion products that have fungicidal/herbicidal properties. Developing next generation control methods using genomic and biotechnological approaches has great potential for control options that will augment and potentially limit the use of synthetic, single-site mode of action, fungicides.

V. Summary and Conclusions

Secretion in phytopathogenic fungi consists of complex molecular pathways and cellular processes that drive virulence. Phytopathogens contain specific receptors that allow them to recognize viable hosts and initiate secretory pathways that lead to the secretion of hundreds of proteins and small molecules from intracellular compartments to the exterior of the cell. The effectors, mycotoxins, and CWDE secreted by phytopathogenic fungi are some of the main determinants of virulence and pathogenic success. The phytopathogenic fungal secretome, as well as the pathways and regulators of secretion, are key avenues of research that can shed light on novel mechanisms that can contribute to limiting the impact of mycotoxins and phytopathogens through the targeted development of new antifungals. Breakthroughs and technological advances over the past 20 years suggest that next generation controls, based on secretion products, could become commercially available in the near future. We anticipate that products and genes of the phytopathogen secretome will be exploited to benefit society in many ways such as the development of next generation biological controls and in synthetic biology to develop targeted metabolic factories. In addition, an understanding of the factors, pathways and regulators involved in secretion will uncover novel

aspects of the pathogen secretome and plant-pathogen interactions to contribute to the larger body of fundamental scientific literature.

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Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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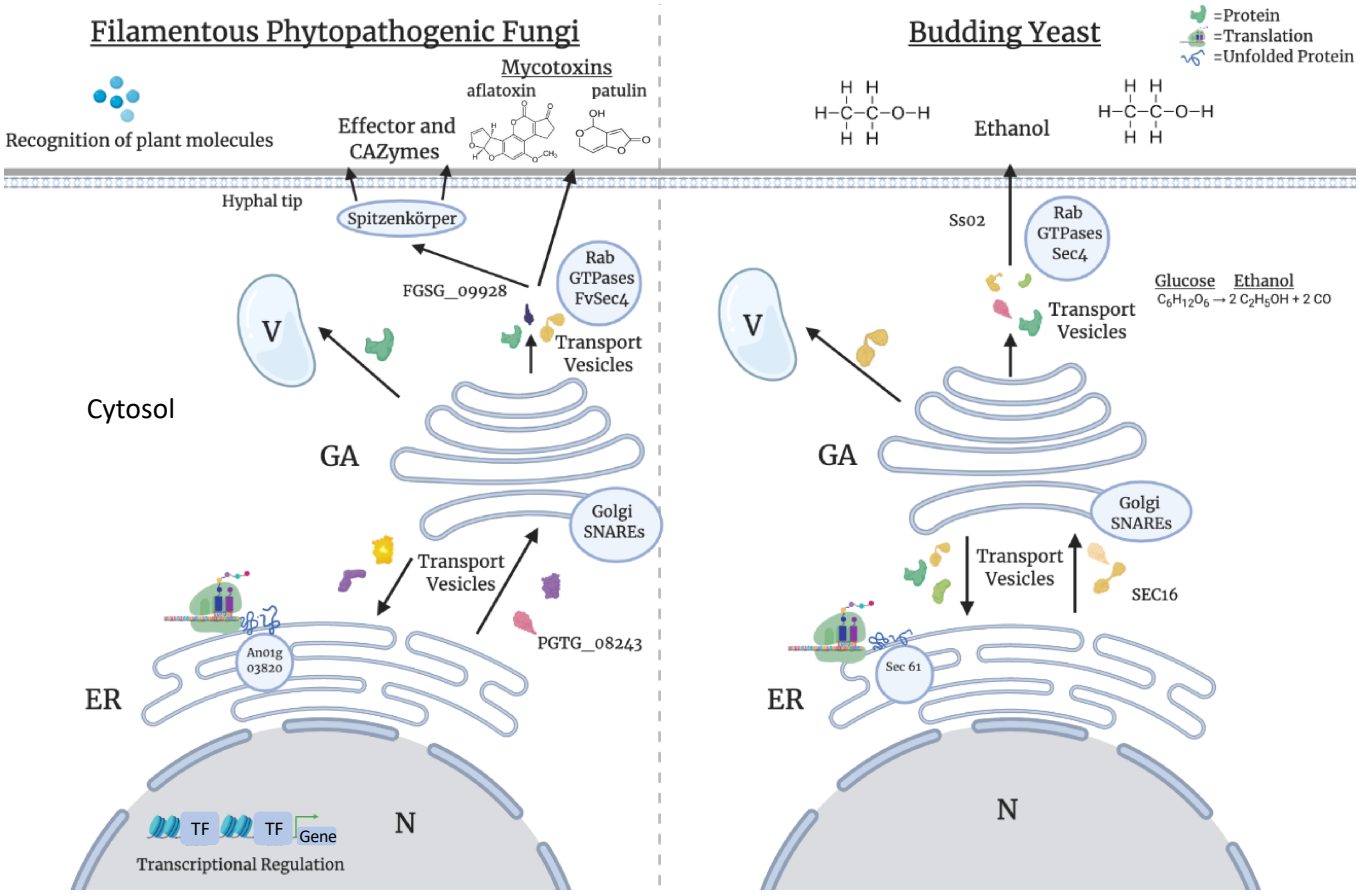

Figure 1. Diagram of the conventional protein secretion pathways in phytopathogenic fungi (left) and budding yeast (right). The pathway required for protein secretion is conserved between phytopathogens and budding yeast and share many homologs in common. Proteins are synthesized in the cytosol and then co-translationally translocated to the endoplasmic reticulum where they are glycosylated or decorated with other carbohydrate/lipid moieties. Proteins are transported by vesicles to the Golgi and then to the exterior of the cell, typically requiring a signal peptide to direct the protein to the proper cellular destination. Proteins can also be transported to the vacuole for degradation. A few genes and proteins involved in the secretory system are shown in budding yeast as well as their corresponding homologs in the filamentous phytopathogens *Aspergillus niger* (Ano1g03820), *Fusarium graminearum* (FGSG_09928), *Fusarium verticilliodes* (FvSec4) or *Puccinia graminis*

(PGTG_08243). N=Nucleus, V=Vacuole, ER=Endoplasmic Reticulum, GA=Golgi Apparatus, TF=Transcription Factor. Figure was made using BioRender.com.

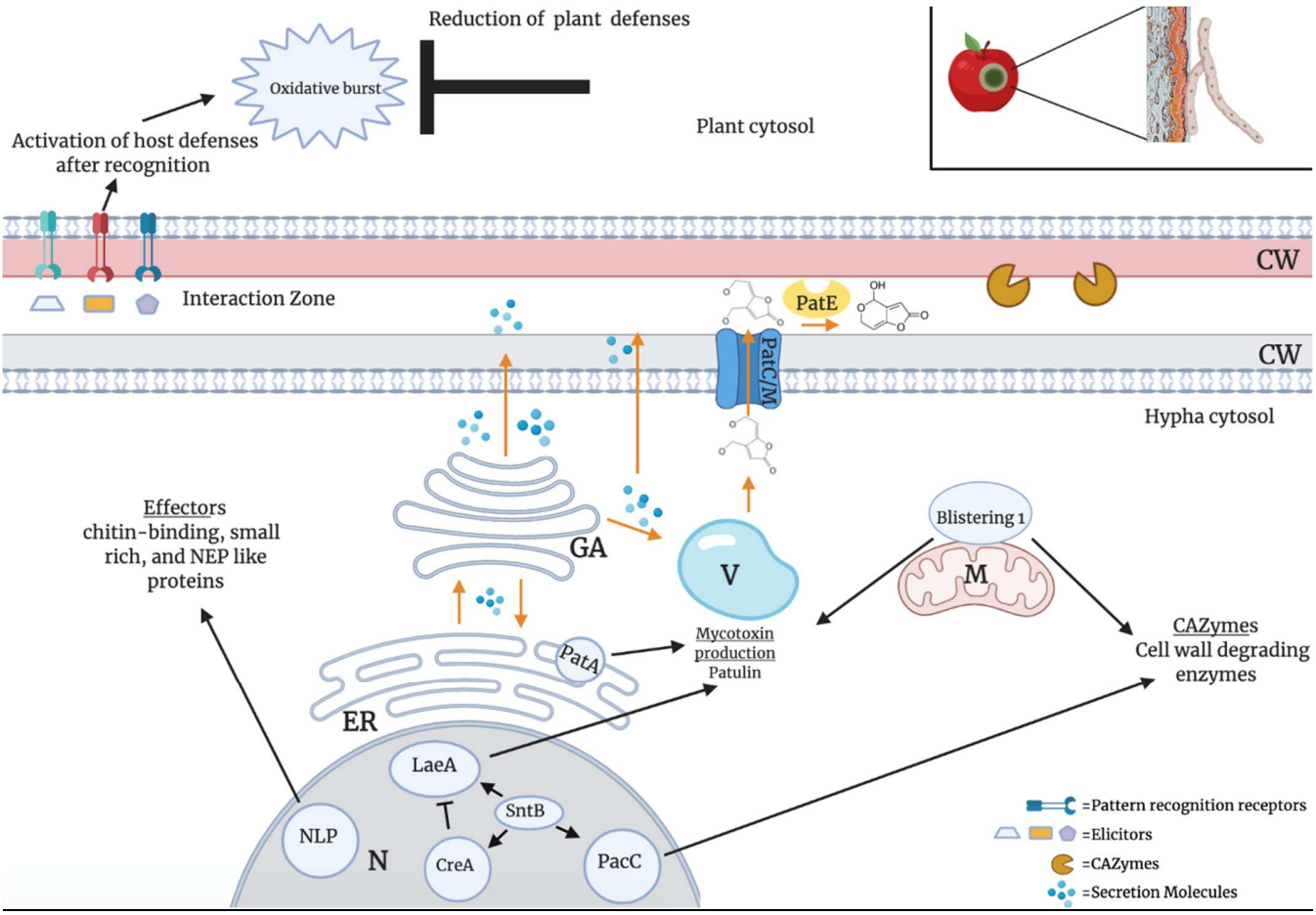

Figure 2. Current conceptual model displaying the secretory molecules and machineries in the *Penicillium expansum-Malus domestica* system that are associated with virulence. A focus on the genes, and the pathway, for the secretion of the mycotoxin patulin is presented. Figure was made using BioRender.com. Image of the apple cells in the upper right corner originate from Lashbrooke et al. (2015). Black arrows from a gene refers to the secretory product the gene is associated with whereas orange arrows signify the movement of molecules through the secretory machinery. N=Nucleus, V=Vacuole, ER=Endoplasmic Reticulum, GA=Golgi Apparatus, M=Mitochondrion.

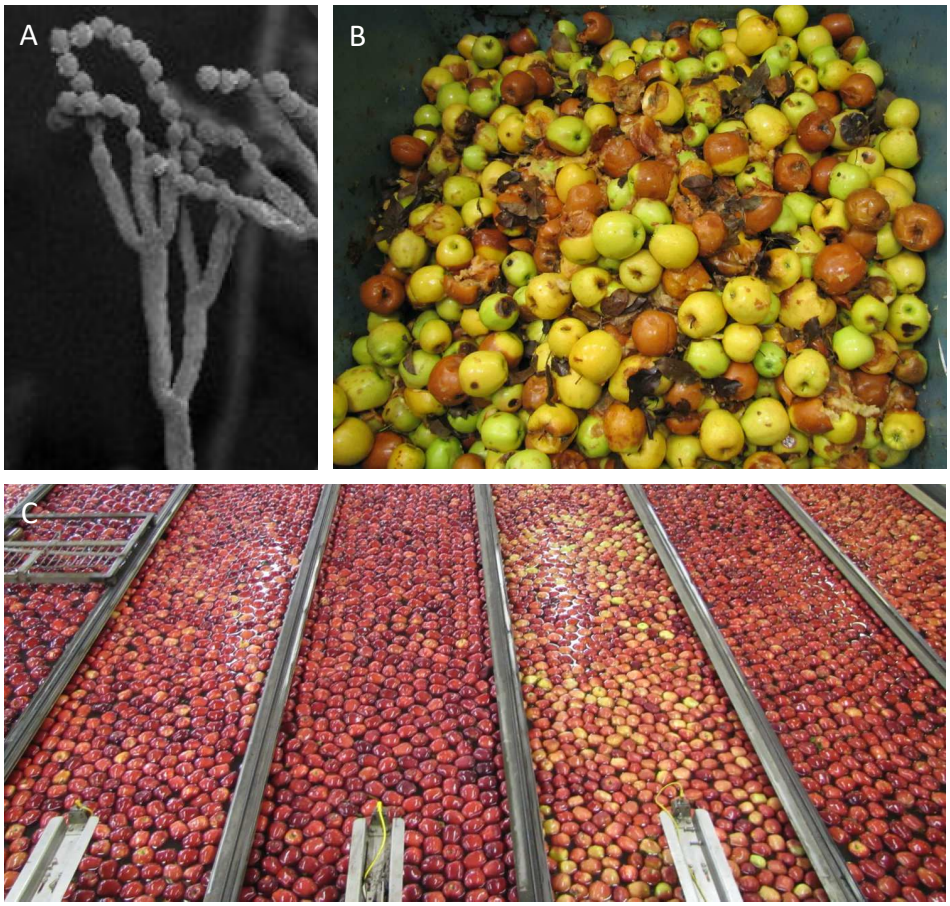

Figure 3. Mycotoxin producing fungi impact the global agricultural industry. A) Scanning electron micrograph showing whorled conidiophore containing spherical conidia produced terminally in chains from one of the main mycotoxin producing fungi, *Penicillium expansum*. B) Apple cull pile consisting of a range of decayed fruit containing mycotoxin producing fungi at a commercial apple packing and storage facility. C) Sorting and grading high quality apple fruit prior to fungicide application via drench at a commercial apple fruit packer.