Defeated by the nines: nine extracellular strategies to avoid microbe-associated molecular patterns recognition in plants

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Abstract
Recognition of microbe-associated molecular patterns (MAMPs) by cell-surface receptors is pivotal in host-microbe interactions. Both pathogens and symbionts establish plant-microbe interactions using fascinating intricate extracellular strategies to avoid recognition. Here we distinguish nine different extracellular strategies to avoid recognition by the host, acting at three different levels. To avoid the accumulation of MAMP precursors (Level 1), microbes take advantage of polymorphisms in both MAMP proteins and glycans, or downregulate MAMP production. To reduce hydrolytic MAMP release (Level 2), microbes shield MAMP precursors with proteins or glycans and inhibit or degrade host-derived hydrolases. And to prevent MAMP perception directly (Level 3), microbes degrade or sequester MAMPs before they are perceived. We discuss examples of these nine strategies and envisage three additional extracellular strategies to avoid MAMP perception in plants.

Introduction
Plants are a rich source of nutrients for many organisms (Cardinale et al., 2011). Their root and aerial systems are exposed to a wide range of microbes including bacteria, fungi, and oomycetes. Some of these microbes are plant pathogens that cause detrimental effects on plant fitness (Dangl and Jones, 2001; Dodds and Rathjen, 2010), while symbiotic microbes can promote plant growth, such as rhizobacteria and arbuscular mycorrhizal fungi (Pérez-de-Luque et al., 2017).

When microbes enter the extracellular space within plant tissues (the apoplast), they interact with host cells that carry surface receptors that recognize conserved molecules, conventionally called microbe-associated molecular patterns (MAMPs, Jones and Dangl, 2006). MAMPs are fragments of proteins or glycans that are essential for the biology of microbes and are absent in the host plant. Examples are fragments of flagellin and peptidoglycans (PGN) from bacteria and fragments of chitin and β-glucans from fungi and oomycetes. These MAMPs are recognized on the host cell surface by pattern recognition receptors (PRRs). PRRs are transmembrane receptor-like kinases (RLKs) or receptor-like proteins (RLPs) that often carry extracellular leucine-rich repeat (LRR) or lysine motif (LysM) domains to confer MAMP recognition (Couto and Zipfel 2016; Tang et al., 2017; Boutrot and Zipfel, 2017; Schellenberger et al., 2019). PRR activation triggers a series of immune responses (Bigeard et al., 2015), resulting in pattern-triggered immunity (PTI) and preventing colonization by nonadapted microbes (Bigeard et al., 2015; Andersen et al., 2018). Importantly, many MAMPs require hydrolytic release from their precursors before they...
can be perceived by PRRs. For instance, hydrolytic release from precursors is required for the recognition of flagellin and elongation factor Tu (EF-Tu), and for the recognition of chitin and β-glucan.

To colonize plants, adapted pathogens and symbionts have adopted strategies to avoid recognition by the immune system. Many effector proteins that act inside the host cell interfere in signaling downstream of PRRs (Toruño et al., 2016). In this review, however, we describe nine extracellular strategies that plant pathogens and symbionts use to avoid recognition by PRRs (Table 1). These strategies occur at three levels: MAMP production (Figure 1A), MAMP release (Figure 1B), and MAMP perception (Figure 1C).

**Level 1: Three strategies Preventing MAMP production**

We distinguish three different strategies that microbes use to prevent the accumulation of MAMP precursors (Level 1, Figure 1A). The first strategy is to accumulate mutations in protein-based MAMPs to avoid recognition of the MAMP fragment. The second strategy is a similar genetic adaptation to alter glycan-based MAMPs beyond recognition. The third strategy is to downregulate the accumulation of MAMP precursors upon infection. The latter can involve transcription factors, epigenetic regulation, and posttranscriptional control. Specific examples of the three strategies are illustrated in Figure 2.

**Strategy 1: Polymorphisms in protein MAMPs**

Polymorphisms in MAMP protein sequences are a frequently used strategy to avoid detection. Sequence polymorphisms have been described for MAMPs from bacterial flagellin, EF-Tu, and RaxX.

In most angiosperms, recognition of bacterial flagellin is mediated by Flagellin Sensing 2 (FLS2), a PRR with extracellular LRRs (Gómez-Gómez and Boller, 2000). FLS2 recognizes peptides from a highly conserved 22-amino acids region in the N-terminal domain of flagellin, called flg22 (Felix et al., 1999; Zipfel et al., 2004). However, some flagellated bacteria carry flg22 sequences that are not recognized by FLS2. flg22 peptides from most ε- and θ-proteobacteria induce moderate, weak, or no response, respectively, in contrast to flg22 peptides from the majority of γ- and β-proteobacteria, which trigger strong responses (Cheng et al., 2020). For instance, flagellin of the crown gall disease pathogen Agrobacterium (Agrobacterium tumefaciens, an α-proteobacterium) possesses a different flg22 sequence that does not trigger FLS2-mediated immune responses in most plant species including Arabidopsis (Arabidopsis thaliana; Felix et al., 1999). However, some γ- and β-proteobacteria also evade FLS2 recognition with sequence polymorphism. For example, the bacterial wilt pathogen Ralstonia solanacearum (a β-proteobacterium) and some strains of Xanthomonas campestris pathovar (pv.) campestris (Xcc; a γ-proteobacterium) have nonrecognizable versions of the flg22 sequence that evade FLS2-mediated defenses in their respective hosts (Pfund et al., 2004; Sun et al., 2006; Wei et al., 2018). Specifically, an aspartate (D) to valine (V) substitution at amino acid position 14 within the flg22 sequence of flagellin from Xcc results in reduced immune responses associated with increased virulence of Xcc on Arabidopsis (Figure 2A; Sun et al., 2006). Similarly, X. oryzae pv. oryzae (Xoo) and pv. oryzicola (Xoc) evade rice (Oryza sativa) FLS2-mediated recognition with substitutions in the flg22 sequence (Wang et al., 2015). Consistent with selection for immune evasion, nonpathogenic strains of X. arboricola pv. juglandis carry the conserved flg22 sequence whereas pathogenic strains carry polymorphisms within the flg22 sequence that evades recognition by FLS2 (Cesbron et al., 2015). Evasion of flagellin recognition by flg22 polymorphisms is also observed with symbiotic bacteria. For instance, the plant beneficial endophytic bacterium Burkholderia phytofirmans (a β-proteobacterium) and the plant beneficial bacterium Sinorhizobium meliloti (an α-proteobacterium) carry flg22 sequences with weak elicitor activity in grapevine (Vitis vinifera) and with no elicitor activity, respectively (Felix et al., 1999; Trdá et al., 2014).

Flagellin is also recognized by FLAGELLIN-SENSING 3 (FLS3), which is present only in some solanaceous plant species including some cultivars of tomato (Solanum lycopersicum), potato (S. tuberosum), and pepper (Capsicum annuum; Hind et al., 2016). FLS3 recognizes flgll-28, a 28-amino acid peptide from the central region of the flagellin protein. Interestingly, polymorphisms within flgll-28 sequences are observed between strains of Pseudomonas syringae pv. tomato (Pto; a γ-proteobacterium). Flagellin of the Col338 strain of Pto contains a valine (V) residue at position 13 in the flgll-28 sequence, and this peptide triggers a weaker immune response in tomato than the flgll-28 peptide from the PtoT1 strain, which carries an alanine (A) residue at this position (Cai et al., 2011).

Evasion of recognition caused by flagellin polymorphisms has also been described for animal pathogens. For instance, the human pathogenic bacteria Campylobacter jejuni, Helicobacter pylori, and Bartonella bacilliformis evade flagellin recognition by Toll-Like Receptor 5 (TLR5) with mutations within the key recognition sites of the flagellin N-terminal D1 domain (Andersen-Nissen et al., 2005).

Besides flagellin, evasion with protein polymorphism has also been reported for peptides containing the first 18 amino acids of bacterial Elongation Factor Thermal unstable (EF-Tu), called elf18, which is recognized in Arabidopsis by the EF-Tu Receptor (EFR; Kunze et al., 2004; Zipfel et al., 2006). Polymorphism within the elf18 sequence correlates with different elicitation activity. For instance, elf18 peptides from Xcc and PtoDC3000 trigger only 0.8%–3.2% of the immune activity as compared to peptides from Agrobacterium, Ralstonia, and other Xanthomonas and Pseudomonas strains (Lacombe et al., 2010).

Another bacterial MAMP with protein polymorphism is the tyrosine-sulfated peptide RaxX, which is perceived by the rice immune receptor XA21 (Pruitt et al., 2015; Luu et al., 2019). XA21 confers resistance to most strains of X. oryzae pv. oryzae (Xoo; Wang et al., 1996). However, Xoo
Table 1. Overview of strategies employed by microbes to evade MAMP recognition in plants

| Microbe                           | MAMP or MAMP precursor | Microbial protein | Plant host | Host target (PRR) | References                        |
|-----------------------------------|------------------------|-------------------|------------|-------------------|-----------------------------------|
| **Bacteria**                      |                        |                   |            |                   |                                   |
| Agrobacterium tumefaciens         | flg22                  | N/A               | Arabidopsis|                   | Felix et al. (1999)               |
| Ralstonia solanacearum            |                        |                   | Arabidopsis|                   | Pfund et al. (2004); Wei et al. (2018) |
| Xanthomonas campestris pv. campestris |              |                   | Arabidopsis|                   | Sun et al. (2006)                 |
| Xanthomonas oryzae pv. and pv. oryzicola |             |                   | rice       |                   | Wang et al. (2015)                |
| Xanthomonas arboricola pv. juglandis |          |                   | juglans regia|                 | Cesbron et al. (2015)             |
| Sinorhizobium meliloti             |                        |                   | tomato, rice|                   | Felix et al. (1999)               |
| Burkholderia phytofirmans         |                        |                   | grapevine  |                   | Trdi et al. (2014)                |
| Pseudomonas syringae pv. tomato   | fglIl-28               | N/A               | Arabidopsis| (FLS3)            | Cai et al. (2011)                 |
| Xanthomonas campestris pv. campestris and Pseudomonas syringae pv. tomato |       |                   | tomato     | (EFR)             | Lacombe et al. (2010)             |
| Xanthomonas oryzae pv. oryzae     |                        |                   | rice       | (Xa21)            | Pruitt et al. (2015)              |
| **Fungi**                         |                        |                   |            |                   |                                   |
| Verticillium dahliae              | Chitin                 | VdPDA1            | cotton     |                   | Gao et al. (2019)                 |
| Puccinia striiformis f. sp. tritici |              | Pst13661         | wheat      |                   | Xu et al. (2020)                  |
| Puccinia graminis f. sp. tritici  |                        |                   | wheat      |                   | El Gueddari et al. (2002)         |
| Uromyces fabae                    |                        | N/A               | broad bean |                   |                                   |
| Colletotrichum graminicola        |                        |                  | maize      |                   |                                   |
| Pestalotiopsis sp.               |                        | PesCDa            | rice       |                   |                                   |
| Colletotrichum graminicola        | β-glucan               | KRES and KRE6     | N/A        |                   | Cord-Landwehr et al. (2016)        |
| Cladosporium fulvum              | Chitin                 | Avr4              | tomato     |                   |                                   |
| Verticillium nonalfalfae          |                        | VnaCheBP          | hop (Humulus lupulus L.)| (CERK1)  | Van den Burg et al. (2006)         |
| Colletotrichum higginsianum       |                        | VdLysM            | tomato     |                   | Volk et al. (2019)                |
| Parastagonospora nodorum          |                        | ChELP1 and ChELP2| monocot and dicot crops |                   | Kombrink et al. (2017)            |
| Clonostachys rosea                |                        | SnTox1            | wheat      |                   | Takahara et al. (2016)            |
| Serendipita indica                |                        | LysM1 and LysM2   | Arabidopsis, barley, and N. benthamiana |       | Liu et al. (2016)                 |
| **Fungi**                         |                        | β-glucan          | maize      |                   | Dubey et al. (2020)               |
| Pseudomonas syringiae pv. tabaci 6605 |              | flgellin glycan  | tobacco    | N/A               | Wawra et al. (2016)               |
| Xanthomonas campestris pv. campestris XcA  |          |                   | soybean    |                   |                                   |
| Acidovorax avenue K1 strain      |                        |                   | cabbage    |                   |                                   |
| Xanthomonas campestris pv. syringae B728a |          |                   | rice       |                   |                                   |
| Xanthomonas campestris pv. syringae B728a |          |                   | N. benthamiana |                   |                                   |

(continued)
| Microbe                        | MAMP or MAMP precursor | Microbial protein | Plant host | Host target (PRR) | References                  |
|-------------------------------|------------------------|-------------------|------------|-------------------|----------------------------|
| Xylella fastidiosa            | LPS                    | LPS glycan        | grapevine  | N/A               | Rapicavoli et al. (2018)   |
| Pseudomonas syringae pv. syringae B728a |                        |                   | bean       |                   | Helmann et al. (2019)      |
| Magnaporthe oryzae            |                        |                   |            |                   | Fujikawa et al. (2009)     |
| Cochlioborus miyabeanus       | Chitin                 | α-1,3-glucan      | rice       | N/A               | Fujikawa et al. (2012)     |
| Rhizoctonia solani            |                        |                   |            |                   |                            |

**Strategy 6: Blocking MAMP release by inhibiting the activity of host hydrolases**

| Oomycete                     | β-glucan               |                   |            |                   | Rose et al. (2002)         |
| Phytophthora sojae           |                        |                   |            |                   | Damasceno et al. (2008)    |
| Phytophthora infestans       |                        |                   |            |                   | Wang et al. (2021)         |
| Phoma herbarum               |                        |                   |            |                   |                            |

**Strategy 7: Disintegrating host-derived hydrolases**

| Fungi                         | Chitin                 |                   |            |                   |                            |
| Fusarium oxysporum f. sp. lycopersici | Chitin | Chitin | tomato | Chi28 | Han et al. (2019) |
| Fusarium verticillioides      |                        |                   |            |                   | Naumann et al. (2011)      |
| Colletotrichum graminicola    |                        |                   |            |                   | Sanz-Martín et al. (2016)  |
| Colletotrichum cinereus       |                        |                   |            |                   | Lilly et al. (2008)        |
| Ustilago maydis               |                        |                   |            |                   | Ökmen et al. (2018)        |

**Level 3: Preventing MAMP perception**

**Strategy 8: Degrading MAMPs**

| Fungi                         | Chitin                 |                   |            |                   |                            |
| Podosphaera xanthii           | Chitin                 |                   |            |                   |                            |
| Podosphaera syringae pv. tomato | Flagellin/flg22   | AprA              | Arabidopsis| (FLS2)     | Baro et al. (2011)         |

**Strategy 9: Sequestering released MAMPs**

| Fungi                         | Chitin                 |                   |            |                   |                            |
| Cladosporium fulvum           | Chitin                 | Ecp6              | tomato     | (FLS2)            | De Jonge et al. (2010)     |
| Mycosphaerella graminicola    |                        | Mg3LysM           | wheat      |                   | Marshall et al. (2011)     |
| Magnaporthe oryzae            |                        | Sip1              | rice       |                   | Mentel et al. (2012)       |
| Verticillium dahliae strain VdLs17 | Vd2LysM           |                   |            |                   | Kombrink et al. (2017)     |
| Colletotrichum higginsianum   | ChELP1 and ChELP2     |                   |            |                   | Takahara et al. (2016)     |
| Rhizoctonia solani            | RSLsM                  |                   |            |                   | Dölfers et al. (2019)      |
| Trichoderma atroviride        | Ta6                    |                   | Arabidopsis and tomato |       | Romero-Contreras et al. (2019) |
| Rhizopathus irregularis       | RSLM                   |                   | Medicago truncatula |       | Zeng et al. (2020)         |
| Monilinia phthora perniciosa  | MIpCh                  |                   | cacao      |                   | Fiorini et al. (2018)      |
| Magnaporthe oryzae            | MChia1                 |                   | rice       |                   | Yang et al. (2019)         |

**Bacteria**

| Bacillus subtilis             | Flagellin              | BSn5              | Arabidopsis and voodoo lily | (FLS2) | Deng et al. (2019) |

See Supplemental Data Set S1 for a version of this table in excel format.
N/A, nonapplicable.
isolates carrying nonsynonymous substitutions of tyrosine residue Y41 in RaxX evade XA21-mediated immunity (Pruitt et al., 2015).

In conclusion, protein MAMP polymorphism is an efficient and frequently used strategy to evade host immunity, employed by both pathogenic and symbiotic bacteria. The polymorphisms in MAMPs highlight the exceptional genetic plasticity associated with host adaptation of bacteria. However, amino acid sequence polymorphism is of course restricted by protein function. Flagellin, for instance, cannot accept many substitutions in the flg22 sequence without affecting flagellin function because this region acts as a hinge that facilitates important conformational changes in the flagellin structure when reversing the spin of flagellar rotation (Fliegmann and Felix, 2016; Wang et al., 2017).
is relevant for protein-based MAMPs, different strategies are
needed for nonproteinaceous MAMPs.

Strategy 2: Polymorphisms in glycan MAMPs
Glycan polymorphism is the second strategy used by micro-
organisms to evade host immunity. This strategy is illus-
trated here by the deacetylation of chitin by fungi.

Many fungi evade host immunity by deacetylating chitin into
chitosan. Chitin is a structural element of fungal cell
walls and chitin fragments are conserved MAMPs that are
almost universally recognized in the plant kingdom by
Chitin Elicitor Receptor Kinase 1 (CERK1) as a signature of
fungal invasion (Pusztahelyi, 2018). Chitosan, however, indu-
ces a weaker immune response than chitin, so the deacetyla-
tion of chitin is frequently used by plant-associated fungi to
avoid recognition (Vander et al., 1998). The soil-borne path-
genic fungus Verticillium dahliae
regulate flagellin biosynthesis during infection. Biosynthesis
for both bacteria and fungi.

Strategy 3: Downregulating MAMP production
Another microbial strategy to evade host detection is to re-
duce the amount of MAMPs by downregulating their bio-
synthesis during infection. This strategy has been described
for both bacteria and fungi.

Pathogenic, opportunistic, and commensal bacteria down-
regulate flagellin biosynthesis during infection. Biosynthesis
of flagella in Pseudomonas is downregulated by the second
mesenger cyclic-di-GMP (cdG; Hickman and Harwood,
2008). Elevated cdG levels in the plant pathogen P. syringae,
the plant opportunist P. aeruginosa and the plant commen-
sal P. fluorescens reduce flagellin levels, and thus contribute
to the evasion of FLS2-mediated immune response in
Nicotiana benthamiana and Arabidopsis (Pfeilmeier et al.,
2016). Flagellin protein levels are also downregulated in
PtoDC3000 via reduction in flagellin expression by the gene
expression regulator AlgU to avoid host immune responses
(Bao et al., 2020).

Downregulation of flagellin genes is also observed upon en-
try of P. syringae pv. syringae B728a (PsyB728a) into the leaf
of bean (Phaseolus vulgaris) plants (Yu et al., 2013).

In fungal pathogens, downregulation of β-glucan biosyn-
thesis reduces exposure to glycan MAMPs. For instance, dur-
ing the biotrophic phase of infection, the fungal maize
pathogen C. graminicola downregulates the expression of
genes encoding Killer toxin resistant 5 (KRES) and KRE6,
which are key enzymes for the biosynthesis of β-glucan
(Figure 2C; Oliveira-Garcia and Deising, 2016). However,
KRES and KRE6 expressions are indispensable for the forma-
tion of appressoria and necrotrophic hyphae. Consistent
with a need for β-glucan, KRE genes are also required for
full virulence of fungal human pathogens C. neoformans and
Candida albicans (Herrero et al., 2004; Gilbert et al., 2010).

In conclusion, downregulating MAMP precursor levels is a
common strategy used by both fungi and bacteria to avoid
host detection. Obviously, this strategy is only beneficial for
the microbe when production of the MAMP precursor is
not required for full virulence. For instance, bacterial prolif-
eration and spread do not rely on flagellin after host entry
and the altered fungal cell wall composition may no longer
need β-glucans upon host entry.

Level 2: Preventing MAMP release
MAMPs discussed in this section are released from microbes
by host-secreted hydrolases, such as chitinases and proteases.
We distinguish four strategies to block MAMP release from
microbes (Figure 1B). MAMP precursors are protected against
host hydrolases by microbial proteins and glycans, and host
hydrolases are also inhibited and disintegrated. Specific ex-
amples of the four strategies are illustrated in Figure 3.

Strategy 4: Hiding MAMP precursors with proteins
Microbial organisms can evade host recognition by secreting
proteins that cover MAMP precursors to prevent hydrolytic
release of MAMPs. There are seven examples of this strategy, involving structurally unrelated secreted proteins used by both pathogenic and symbiotic fungi.

The tomato leaf mold fungus \textit{(Cladosporium fulvum} syn. \textit{Passalora fulva}) produces Avr4, a member of Carbohydrate-binding module family 14 (CBM14). Avr4 specifically binds to chitin in the fungal cell wall to protect it against plant chitinases, which release chitin elicitors (Van den Burg et al., 2006; Figure 3A). Homologs of Avr4 are present in other pathogenic fungi, indicating a similar protection of chitin cell walls by other fungi (Stergiopoulos et al., 2010).

Likewise, the hemibiotrophic xylem-invading fungus \textit{V. nonalfalfae} prevents chitin hydrolysis by secreting VnaChtBP, a CBM18 protein that binds chitin and suppresses chitin-triggered host immunity (Volk et al., 2019). VnaChtBP is present in 28 \textit{V. nonalfalfae} isolates, suggesting a high evolutionary stability and testifying its importance for the fungal lifestyle.

Similarly, the fungal vascular wilt pathogen \textit{V. dahliae} strain VdLs17 secretes the lineage-specific LysM effector VdLysM, a CBM50 protein (Akcapinar et al., 2015), which binds chitin, suppresses chitin-induced immune responses, and protects fungal hyphae against hydrolysis by plant hydrolytic enzymes (Kombrik et al., 2017). Likewise, the causative fungus of anthracnose diseases, \textit{C. higginsianum}, produces the extracellular LysM proteins 1 and 2 (ChELP1 and ChELP2), which bind chitin and prevent chitin-triggered immunity (Takahara et al., 2016).

The wheat Septoria nodorum blotch (SNB) pathogen \textit{Parastagonospora nodorum} secretes SnTox1, a protein that also binds chitin and protects the pathogen from wheat chitinases (Liu et al., 2016). Interestingly, SnTox1 also induces host cell death, supporting the necrotrophic lifestyle of this pathogen (Liu et al., 2012).

Plant beneficial fungi that are parasites of pathogenic fungi also avoid plant immune response by covering MAMPs with proteins. For example, the mycoparasite \textit{Clonostachys rosea} (syn. \textit{Gliocladium roseum}) produces CBM50 members LysM1 and LysM2 to protect hyphae against chitinases to prevent MAMP-induced defenses during wheat root colonization by its host \textit{F. graminearum} (Dubey et al., 2020).

Fungi also prevent MAMP release by hiding \(-\text{glucans with proteins. The root endophyte Serendipita indica} (\textit{Si}, \textit{Syn. Piriformospora indica}), avoids recognition of its \(-\text{glucan by secreting a fungal-specific \(-\text{glucan-binding lectin, Fungal Glucan-Binding 1} (\text{FGB1})\). SiFGB1 binds \(\text{-glucan to reduce \(\text{-glucan-triggered immunity in several host plants, including Arabidopsis, barley (Hordeum vulgare), and N. benthamiana (Wawra et al., 2016).}\)}\)

In conclusion, covering MAMP precursors with proteins to prevent their hydrolysis is a mechanism used by many fungal pathogens and symbionts. Remarkably, several structurally unrelated carbohydrate-binding proteins (Avr4, ChEtBP, Vd2LysM, ChELP1 and ChELP2, SnTox1, LysM2, and FGB1) have convergently evolved to protect fungal hyphae from hydrolysis by plant chitinases and glucanases, which would otherwise release MAMP from their precursors. In addition to preventing MAMP release, these proteins can also promote virulence by strengthening the cell wall.

\textbf{Strategy 5: Shielding MAMP precursors with glycans}

Glycosylation of MAMP precursors to shield them from hydrolytic release of MAMPs is the fifth strategy to evade host...
faster immune responses (Rapicavoli et al., 2018). In O- and an oligosaccharide core region that carries an lipid A, a di-glucosamine carrying four to seven fatty acids, outer membrane of Gram-negative bacteria and consist of to evade immunity. LPSs are the major component of the gests that different glycoforms on flagellin are required for may alter host responses. For instance, the plant pathogenic bacterial species and strains. Glycans covering bacterial LPS ride repeats. OPS composition is highly diverse among charide (OPS) comprising a variable number of oligosaccha-
lease of the flagellin MAMP (Figure 3B). For instance, glycan (Yamamoto et al., 2011; Chiku et al., 2013). This sug-
hydrolysis by BGAL1 (Buscaill et al., 2019), even though are absent from Psy (Segonzac et al., 2011). Importantly, mVio biosynthesis genes (Takeuchi et al., 2003; Taguchi et al., 2006; Ichinose et al., 2013). Flagellin glycosylation is also important for Acidovorax avenae, a Gram-negative bacterial pathogen causing leaf blight in rice. Flagellin isolated from the avirulent N1141 strain induces immune responses, whereas flagellin from the virulent K1 strain does not. These flagellin protein sequences are identical but their glycosylation pattern is different: strain K1 carries a 2,150-Da O-glucan while strain N1141 harbors a 1,600-Da O-glycan (Hirai et al., 2011). Thus, glycosylation avoids flagellin recogni-
tion, presumably by preventing the hydrolytic release of immunogenic flagellin fragments.

Consistent with shielding glycans, plant-secreted β-galactosidase 1 (BGAL1) acts in immunity by promoting the release of immunogenic peptides from glycosylated flagellin of PtoDC3000 and P. syringae pv. tabaci 6605 (Pta6605), which both carry a terminal-modified viosamine (mVio) sugar on flagellin O-glycans (Buscaill et al., 2019). BGAL1 is not required to release the flagellin MAMP from the Δfgt1 mutant of Pta6605, which produces nonglycosylated flagellin. Interestingly, pv. syringae B728a (PsyB728a) evades host immunity by having O-glycans on flagellin that are resistant to hydrolysis by BGAL1 (Buscaill et al., 2019), even though PsyB728a has an flg22 sequence recognized by FLS2 (Segonzac et al., 2011). Importantly, mVio biosynthesis genes are absent from PsyB728a, which instead carries a putative (1,2)-linked terminal GlcNac (N-acetylgalacosamine) on its O-
glycan (Yamamoto et al., 2011; Chiku et al., 2013). This sug-
gests that different glycoforms on flagellin are required for the colonization of different hosts and that hosts may use different glycosidases to release flagellin MAMPs.

Bacteria also use polymorphic lipopolysaccharides (LPSs) to evade immunity. LPSs are the major component of the outer membrane of Gram-negative bacteria and consist of lipid A, a di-glucosamine carrying four to seven fatty acids, and an oligosaccharide core region that carries an O-polysac-
charide (OPS) comprising a variable number of oligosaccharide repeats. OPS composition is highly diverse among bacterial species and strains. Glycans covering bacterial LPS may alter host responses. For instance, the plant pathogenic bacterium Xylella fastidiosa possesses a long chain O-antigen that delays recognition by the host plant (Rapicavoli et al., 2018). Mutant X. fastidiosa lacking these O-antigens induces faster immune responses (Rapicavoli et al., 2018). In addition, genes that encode glycosyltransferase domains and cause strong virulence phenotypes when disrupted in PsyB728a are suspected to be involved in the biosynthesis of O-antigens that decorates LPS (Helmann et al., 2019).

Glycosylation of fungal cell walls is also used to prevent MAMP release. For instance, the rice blast fungus Magnaporthe oryzae, the rice brown spot fungus Cochlioborus miyabeanus, and the rice sheath blight fungus Rhizoctonia solani accumulate α-1,3-glucan on the surface of infectious hyphae (Fujikawa et al., 2009, 2012). Fungal mutants with reduced α-1,3-glucan levels have reduced viru-

Strategy 6: Blocking MAMP release by inhibiting the activity of host hydrolases

The inhibition of MAMP-releasing hydrolases is another strategy used by plant pathogens. Examples of this strategy have been identified in oomycetes and bacterial pathogens. *Phytophthora* species secrete glucanase inhibitor proteins (GIPs) during invasion of their hosts, which themselves inhibit MAMP release through extracellular Endo-β-1,3-Glucanases (EGases). For example, *P. sojae* secretes GIP1 to inhibit soybean EGaseA, thereby preventing EGaseA-mediated release of elicitor-active glucan oligosaccharides (Figure 3C; Rose et al., 2002). Analysis of tomato leaves inoculated with *P. infestans* showed that *P. infestans* GIPs and tomato EGases form stable complexes in the apoplast (Damasceno et al., 2008), indicating that GIPs-mediated inhibition of EGases to prevent MAMP release is a common strategy used by *Phytophthora* in different hosts.

*Phytophthora* species also produce Kazal-like Extracellular Protease Inhibitors (EPIs) during infection. *P. infestans* EPI1 and EPI10 inhibit the secreted subtilisin-like protease P69B of tomato (Tian et al., 2004; Tian et al., 2005). P69B releases a fragment from the apoplastic effector PC2 of *P. infestans* that then triggers immune responses and a hypersensitive response (HR) in solanaceous plants (Wang et al., 2021).
Thus, EPI1 might suppress PC2-elicited host immunity by inhibiting the protease that releases the elicitor.

Bacterial pathogens also produce hydrolase inhibitors to prevent MAMP release. For instance, PtoDC3000 produces the small molecule BGAL1 inhibitor galactosyrin (Buscaill et al., 2019). BGAL1 promotes the release of MAMPs from glycosylated flagellin carrying mVio on their O-glycan (see Strategy 5). BGAL1 is suppressed by galactosyrin during infection to prevent the release of immunogenic peptide from flagellin (Buscaill et al., 2019).

GIP1, EPI1, and galactosyrin are just the first examples of pathogen-derived inhibitors targeting MAMP-releasing host hydrolases. Further studies on widespread pathogen-derived inhibitors will probably uncover many more examples. However, in addition to preventing MAMP release, these inhibitors also protect the physical integrity of flagellin and the microbial cell wall by preventing their degradation.

**Strategy 7: Disintegrating host-derived hydrolases**

Destruction of MAMP-releasing host hydrolases is the seventh strategy used by invading microorganisms to evade immunity. We currently know three unrelated classes of proteases from fungal pathogens that disintegrate host chitinases to prevent MAMP release.

The root-infecting fungal pathogen *V. dahlia* produces Secreted Serine Protease 1 (SSEP1, family S8) during the invasion of cotton root cells to hydrolyze cotton Chitinase 28 (Chi28; Figure 3D; Han et al., 2019). Likewise, the vascular wilt pathogen of tomato, *F. oxysporum* f. sp. lycopersici, secretes family-M36 metalloprotease fungalysin FoMep1 and family-S8 subtilisin-like protease FoSep1 to remove the extracellular chitin-binding domain (CBD) from tomato chitinases SJChi1 and SJChi13 (Jashni et al., 2015). Removal of the CBD significantly reduces chitinase and antifungal activity, thereby playing a pivotal role in virulence of *F. oxysporum*. Similarly, the fungal pathogens *F. verticillioides*, *C. graminicola*, and *Ustilago maydis* also secrete family-M36 metalloprotease fungalysins to cleave plant chitinases (*Lilly et al., 2008; Naumann et al., 2011; Sanz-Martín et al., 2016; Ökmen et al., 2018*). Interestingly, animal fungal pathogens also produce fungalysin during infection, presumably with the same objective (*Li and Zhang, 2014*).

In conclusion, SSEP1, FoSep1, FoMep1, and other fungalysins represent different protease families that cleave plant chitinases to prevent MAMP release. The phylogenetic distance between these fungi and proteases indicates that the inactivation of chitinases evolved convergently. Besides preventing MAMP release, these proteases also protect the physical integrity of the cell wall by preserving chitin.

**Level 3: Preventing MAMP perception**

Once MAMPs are released, we know two effective strategies that prevent the perception of MAMPs by PRRs (Figure 1C). One strategy degrades MAMPs before they reach their receptors, the other strategy sequesters MAMPs before they are perceived. Specific examples of the two strategies are illustrated in Figure 4.

**Strategy 8: Degrading MAMPs**

The eighth strategy used by pathogens is based on the targeted degradation of released MAMPs by pathogen-derived proteases. Examples are the bacterial effectors AprA and LasB and fungal effectors with chitinase activity (EWCAs), explained below.

*Pseudomonas* species secrete alkaline protease ArpA, a 50-kD zinc metalloprotease (MEROPS family M10 of clan MA), which prevents flagellin-triggered immune responses by degrading flagellin monomers and flg22 (Figure 4A; *Bordoel et al., 2011*). Flagellin polymers resist degradation by AprA and this preserves the integrity of the flagellum (*Bordoel et al., 2011*). In Arabidopsis, AprA prevents flg22- and flagellin-induced immune responses and delays stomatal closure. AprA of *PtoDC3000* is required for its full virulence on both Arabidopsis and tomato. Interestingly, AprA is widespread among human- and plant-pathogenic bacteria, including the bacterial plant pathogen *P. syringae* and human pathogen *P. aeruginosa*, suggesting a conserved infection mechanism among bacteria.

In addition to AprA, *P. aeruginosa* secretes a second zinc metalloprotease, the 33-kD pseudolysin LasB (MEROPS family M4 of clan MA), which also degrades flagellin and acts in concert with AprA to prevent flagellin-mediated immune recognition (*Casilag et al., 2016*). The production of two different proteases targeting flagellin monomers likely provides *P. aeruginosa* with robust immune suppression mechanisms.

Fungal pathogens also degrade MAMPs during infection. The cucurbit powdery mildew fungus *Podosphaera xanthii*...
releases effectors with chitinase activity (EWCAs) when penetrating melon (Cucumis melo) plant cells to degrade immunogenic chitin oligomers and thereby prevents the activation of chitin-triggered immunity (Martínez-Cruz et al., 2021). Remarkably, EWCA homologs are also widely distributed in plant fungal pathogens but also in fungi that are pathogens of insects, nematodes, and animals, suggesting a conserved infection mechanism among fungi (Martínez-Cruz et al., 2021).

In conclusion, the degradation of MAMPs is an efficient mechanism to avoid recognition but only a few of these proteases have been identified. Additional pathogen-produced proteases that promote virulence (Chandrasekaran et al., 2016; Figaj et al., 2019) may also act by degrading MAMPs. Likewise, pathogen-secreted glycosidases may degrade glycan-based MAMPs. For instance, the human pathogen Histoplasma capsulatum secretes endo-β-1,3-glucanase Eng1 to evade host detection (Garfoot et al., 2016) and many plant pathogens having glycan-based MAMPs secrete glycosidases (Ospina-Giraldo et al., 2010; Murphy et al., 2011; Vermassen et al., 2019; McGowan et al., 2020). Notably, MAMP degradation must require fine regulation of these hydrolases to avoid unspecific or premature degradation of MAMP precursors or inadvertent MAMP release.

Strategy 9: Sequestering released MAMPs

Elicitor sequestration is the ninth strategy used by plant pathogens to evade recognition. In this strategy, pathogen-secreted proteins bind released elicitors to prevent them from binding host receptors. Many fungi secrete proteins to sequester chitin elicitors.

During infection, C. fulvum secretes Extracellular protein 6 (Ecp6), a LysM-containing protein of the CBM50 family that binds chitin fragments. Ecp6 binds these elicitors with greater affinity than the chitin receptor, so C. fulvum evades chitin recognition by using Ecp6 to sequester chitin fragments (Figure 4B; De Jonge et al., 2010). The widespread presence of Ecp6 orthologs suggests that this is a common strategy of many pathogenic fungi to avoid host recognition (De Jonge and Thomma, 2009). Indeed, the fungal wheat pathogen Mycosphaerella graminicola produces Mg3LysM, an Ecp6 homolog, that plays a major role in pathogen virulence on wheat plants by preventing the elicitation of chitin-induced immunity (Marshall et al., 2011). Similarly, the rice blast fungus M. oryzae produces Secreted LysM protein-1 (Slp1), which binds chitin fragments and prevents chitin-triggered immunity (Menttlak et al., 2012). Interestingly, Vd2LysM from V. dahlia and ChELP1 and ChELP2 from C. higginsianum, also sequester chitin oligomers and additionally protect chitin polymers against chitinases (Strategy 4; Takahara et al., 2016; Kombrik et al., 2017).

Symbiotic fungi also use LysM proteins to establish compatible interactions. The endophytic fungus Trichoderma atroviride and the arbuscular mycorrhiza fungus Rhizophagus irregularis, for instance, produce the LysM proteins Tal6 and RfSLM, respectively, to evade extracellular recognition (Romero-Contreras et al., 2019; Zeng et al., 2020).

Surprisingly, even necrotrophic fungi use LysM proteins to evade immunity. For instance, the necrotrophic fungus R. solani, which kills seedlings and causes root rot in a broad range of plant species, secretes RsLysM, which associates with chitin oligomers to prevent early chitin perception during sugar beet (Beta vulgaris) colonization (Dölfors et al., 2019).

LysM proteins are also used by animal fungal pathogens and contribute to fungal virulence during host invasion. The insecticidal fungus Beauveria bassiana secretes the two LysM effectors Blys2 and Blys5 that bind chitin and prevent the activation of immunity in insects (Cen et al., 2017).

Sequestration of chitin fragments is also achieved through different evolutionary paths. The cacao (Theobroma cacao) witches broom disease Moniliophthora perniciosa produces an enzymatically inactive chitinase (MpcChi) that prevents chitin-triggered immunity by sequestering chitin fragments (Fiorin et al., 2018). Remarkably, its sister species M. roren encodes a second, nonorthologous catalytically inactive chitinase (MrChi). MpcChi and MrChi are both highly expressed during the biotrophic phase of infection. Despite lacking chitinolytic activity, both proteins sequester immunogenic chitin fragments. Similarly, the fungal rice pathogen M. oryzae secretes Chitinase 1 (MoChia1) that binds chitin and can prevent immune responses (Yang et al., 2019).

Bacteria also use the sequestration strategy by targeting MAMP precursors. For instance, the endophyte bacterium Bacillus subtilis BSn5 enhances its colonization of Arabidopsis and voodoo lily (Amorphophallus konjac) through minimizing the stimulation of fig22-induced defense by producing the antimicrobial peptide (lantibiotic) subtilomycin, which binds to its own flagellin (Deng et al., 2019). The presence of subtilomycin biosynthesis genes in genomes of other bacteria suggests that flagellin sequestration is a common strategy of endophytic bacteria to adapt to endosphere niches (Deng et al., 2019).

MAMP sequestration is common to fungal and bacterial microbes, but more details remain to be discovered in other invading microorganisms. The success of this strategy depends on the competition between the microbial-derived sequestering protein and the host PRR.

Concluding remarks and perspectives

Successful plant-associated microbes evade extracellular detection by the host immune system. While certain immune evasion mechanisms are used by microbes from diverse kingdoms, other mechanisms have so far only been described for certain microbes. However, it seems unlikely that these strategies are pathogen-specific, prompting us to expect that this is likely to change with further development of the field.

For most of the nine strategies described above, suppression MAMP perception also results in increased stability of the MAMP precursor. It has therefore not always been robustly demonstrated that the observed enhanced virulence
associated with the strategy is caused by evading MAMP recognition or by increased stability of the MAMP precursor. A good way to investigate this experimentally would be to test for enhanced virulence in the absence of the PRR, as this would indicate an important role in the stabilization of the MAMP precursor.

We can think of at least three additional extracellular strategies that would prevent MAMP recognition (Figure 1D). First, MAMP recognition can be blocked by receptor shedding. This has been described for animal pathogens, but not for plant pathogens. For example, the fungal receptor shedding. This has been described for animal pathogens. For example, the fungal respiratory pathogen *Coccidioides posadasi* secretes the Metalloproteinase Mep1 during endospore differentiation. Mep1 digests the Spherule Outer Wall glycoprotein (SOWgp), resulting in the prevention of host recognition mediated by this receptor (Hung et al., 2005). Similarly, the opportunistic pathogen of human lungs *P. aeruginosa* secretes the metalloproteinase LasB, which cleaves the human urokinase-type Plasminogen Activator Receptor (uPAR) through domain-specific endoproteolysis (Leduc et al., 2007). There have been no reports of PRR inactivation by shedding in plant-microbe interactions yet. By contrast, ectodomain shedding of the legume Symbiosis Receptor Kinase (SYMRRK) causes the formation of a signaling complex with Nod Factor Receptor 5 (NFR5, Antolin-Llovera et al., 2014).

A second possible strategy is the use of antagonists. MAMP antagonists would bind PRRs and prevent the binding of MAMPs to their respective receptors and therefore inhibit PRR function. For instance, C-terminal truncations of the flagellin flg22 elicitor can block flg22 perception by FLS2 in tomato (Meindl et al., 2000), but the existence and use of MAMP antagonists during infection remains to be reported.

A third possible strategy is to alter the apoplastic conditions such that MAMP release and/or perception is inhibited. Although this mechanism has not yet been demonstrated, the regulation of hydrolases by pH and redox status would offer opportunities for pathogens to interfere in MAMP release. Interestingly, several plant pathogens secrete homologs of plant regulatory peptides to suppress host immunity. For instance, the fungal wilt pathogen *F. oxysporum* secretes a functional homolog of rapid alkalinization factors (RALFs), peptide hormones that trigger an increase in apoplastic pH and enhances fungal colonization (Masachis et al., 2016; Thyne et al., 2017).

In conclusion, the extracellular detection of MAMPs by plants is an active and exciting research field. The presence of many MAMPs, hydrolytic enzymes, and hydrolase inhibitors implicate a large and mostly unexplored area of research, still holding most of its secrets. Increased understanding of this extracellular battlefield of both animal and plant pathogens will ultimately translate into new strategies for the prevention of infectious diseases.

**Supplemental Data Set S1.** Overview of strategies employed by microbes to evade MAMP recognition in plants.

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**References**

Akcapanov GB, Kappel L, Sezerman OU, Seidl-Seiboth V (2015) Molecular diversity of LysM carbohydrate-binding motifs in fungi. Curr Genet 61: 103–113

Andersen EJ, Ali S, Byamukama E, Yen Y, Nepal MP (2018) Disease resistance mechanisms in plants. Genes 9: 339

Andersen-Nissen E, Smith KD, Strobe KL, Barrett SL, Cookson BT, Logan SM, Aderem A (2005) Evasion of Toll-like receptor 5 by flagellated bacteria. Proc Natl Acad Sci USA 102: 9247–9252

Antolin-Llovera M, Ried MK, Parniske M (2014) Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod factor receptor 5. Curr Biol 24: 422–427

Arora SK, Neely AN, Blair B, Lory S, Ramphal R (2005) Role of motility and flagellin glycosylation in the pathogenesis of *Pseudomonas aeruginosa* burn wound infections. Infect Immun 73: 4395–4398

Bao Z, Wei HL, Ma X, Swingle B (2020) *Pseudomonas syringae* AlgU downregulates flagellin gene expression, helping evade plant immunity. J Bacteriol 202: e00418–e00419

Bardiel BW, van der Ent S, Pel MJ, Tommassen J, Pieterse CM, van Kessel KP, van Strijp JA (2011) *Pseudomonas* evades immune recognition of flagellin in both mammals and plants. PLoS Pathog 7: e1002206

Bigeard J, Colcombet J, Hirt H (2015) Signaling mechanisms in pattern-triggered immunity (PTI). Mol Plant 8: 521–539

Boneca IG, Dussurget O, Cabanes D, Nahori MA, Sousa S, Lecuit M, Psylkinakis E, Bouriotis V, Hugot JP, Giovannini M, et al. (2007) A critical role for peptidoglycan N-deacetylation in Listeria evasion from the host innate immune system. Proc Natl Acad Sci USA 104: 997–1002

Boutrot F, Zipfel C (2017) Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. Annu Rev Phytopathol 55: 257–286

Buscail P, Chandrasekar B, Sanguanakitichai N, Kourelis J, Kaschani F, Thomas EL, Morimoto K, Kaiser M, Preston GM, Ichinose Y et al. (2019) Glysidas and glycan polymorphism control hydrolytic release of immunogenic flagellin peptides. Science 364: 145

Cai R, Lewis J, Yan S, Liu H, Clarke CR, Campanile F, Almeida NF, Studholme DJ, Lindeberg M, Schneider D, et al. (2011) The plant pathogen *Pseudomonas syringae* pv. *tomato* is genetically monomorphic and under strong selection to evade tomato immunity. PLoS Pathog 7: e1002130

Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, Balvanera P, O’Connor MI, Gonzalez A (2011) The...
functional role of producer diversity in ecosystems. Am J Bot 98: 572–592
Casiagli F, Lorenz A, Krueger J, Klawonn F, Weiss S, Häussler S (2016) The LasB Elastase of Pseudomonas aeruginosa acts in concert with alkaline protease AprA to prevent flagellin-mediated immune recognition. Infect Immun 84: 162–171
Cen K, Li B, Lu Y, Zhang S, Wang C (2017) Divergent LysM effectors contribute to the virulence of Beauveria bassiana by evasion of insect immune defenses. PLoS Pathog 13: e1006604
Cesbron S, Briand M, Essaki S, Girodne CM, Bishop JG, Ripoll DR, Win J, Kamoun S, Rose JK (2016) Regulation of pattern recognition receptor response by LysM and NBS-LRR effectors in plants. Cell 166: 1151–1164
Changi S, Binah M, Bhuiyan N, Mehta K, Nishimura M, Zhang S, Wang C (2010) Plant immunity: towards an integrated view of plant-pathogen interactions. Nat Rev Genet 11: 539–548
Chang JHT, Bredow M, Monaghan J, D'Inzeno GC (2020) Proteobacteria encode diverse flag22 peptides that elicit varying immune responses in Arabidopsis thaliana. Mol Plant-Microbe Interact. 10.1094/MPMI-11-20-0314-SC
Chikae K, Yamamoto M, Ohnishi-Kameyama M, Ishii T, Yoshida K, Yamamoto M (2019) Engagement of FPR1 and the contribution of glycosylated flagellin to evasion of human innate immune responses. J Biol Chem 294: 8598–8609
Choudhury SD, Giri R, Nishimura M (2009) Dynamics of cell wall components of Magnaporthe grisea during infectious structure development. Mol Microbiol 73: 553–570
Couto D, Zipfel C (2016) Regulation of pattern recognition receptor signalling in plants. Nat Rev Immunol 16: 537–552
Cullender TC, Chassaing B, Janzon A, Kumar K, Muller CE, Werner JJ, Angenent LT, Bell ME, Hay AG, Peterson DA, et al. (2013) Innate and adaptive immunity interact to quench microbe flagellar motility in the gut. Cell Host Microbe 14: 571–581
Damasceno CM, Bishop JG, Ripoll DR, Win J, Kamoun S, Rose JK (2008) Structure of the glucanase inhibitor protein (GIP) family from phytophthora species suggests coevolution with plant endo-beta-1,3-glucanases. Mol Plant 1: 151–157
Dangl JL, Jones JD (2001) Plant pathogens and integrated defence responses to infection. Nature 411: 826–833
De Jonge R, Thomma B.P.H.J. (2009) Fungal LysM effectors: extinguishers of host immunity? Trends Microbiol. 17: 151–157
De Jonge R, van Esse HP, Kombrink A, Shinya T, Desaki Y, Bours R, van der Krol S, Shibuya N, Joosten MH, Thomma BPHJ (2010) Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science 329: 953–955
De Maayer P, Cowan DA (2016) Comparative genomic analysis of Dolfors F, Holmquist A, Thomazella DPT, do Prado PFV, de Nascimento LC, Figueira AVO, Thomma BPHJ, Pereira GAG, Teixeira PJPL (2018) Suppression of plant immunity by fungal chinase-like effectors. Curr Biol 28: 3023–3030
Fleigmann J, Felix G (2016) Immunity: flagellin seen from all sides. Nat Plants 2: 16136
Fujikawa T, Kuga Y, Yano S, Yoshimi A, Tachiki T, Abe K, Nishimura M (2009) Dynamics of cell wall components of Magnaporthe grisea during infectious structure development. Mol Microbiol 73: 553–570
Fujikawa T, Sakaguchi A, Nishizawa Y, Kouzai Y, Minami E, Yano S, Koga H, Meshi T, Nishimura M (2012) Surface alpha-1,3-galactan regulates fungal stealth infection by interfering with innate immunity in plants. PLoS Pathog 8: e1002882
Gao F, Zhang BS, Zhao HJ, Huang JF, Jia PS, Wang S, Zhang J, Zhou JM, Guo HS (2019) Deacetylation of chitin oligomers increases virulence in soil-borne fungal pathogens. Nat Plants 5: 1117–1126
Garfoot AL, Shaan Q, Wüthrich M, Klein BS, Rappeleye CA (2016) The Eng1 beta-glucanase enhances histoplasma virulence by reducing beta-glucan exposure. mBio 7: e01388–e01315
Gilbert NM, Donlin MJ, Gerik KJ, Specht CA, Djordjevic JT, Wilson CF, Sorrell TC, Lodge JK (2010) KRE genes are required for beta-1,6-galactan synthesis, maintenance of capsule architecture and cell wall protein anchoring in Cryptococcus neoformans. Mol Microbiol 76: 517–534
Griffol-Romero L, Sainz-Polo MA, Albasa-Jove D, Guerin ME, Biarnès X, Planas A (2019) Structure-function relationships underlying the dual. J Biol Chem 294: 19666–19680
Gómez-Gómez L, Boller T (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell 5: 1003–1011
Han LB, Li YB, Wang FX, Wang WY, Liu J, Wu JH, Zhong NQ, Wu SJ, Jiao GL, Wang HY et al. (2019) The cotton apoplastic protein CRR1 stabilizes Chtitinase 28 to facilitate defense against the fungal pathogen. Plant Cell 31: 520–536
Hanuszkiwicz A, Pittcock P, Humphries F, Moll H, Rosales AR, Molinaro A, Moynagh PN, Lajoie GA, Valvano MA (2014) Identification of the flagellin glycosylation system in Burkholderia cenocepacia and the contribution of glycosylated flagellin to evasion of human innate immune responses. J Biol Chem 289: 19231–19244
Helmann TC, Deutschbauer AM, Lindow SE (2019) Genome-wide identification of Pseudomonas syringae genes required for fitness during colonization of the leaf surface and apoplast. Proc Natl Acad Sci USA 116: 18900–18910
Hembach L, Bonin M, Gorzelanny C, Moerschbacher BM (2020) Unique subsite specificity and potential natural function of a chitosan deacetylase from the human pathogen. Proc Natl Acad Sci USA 117: 3551–3559
Herrero AB, Magnelli P, Mansour MK, Levitz SM, Bussey H, Abeijon C (2004) KRES genes null mutant strains of Candida albicans are avirulent and have altered cell wall composition and hypha formation properties. Eukaryot Cell 3: 1423–1432
Hernández-Chávez MJ, Pérez-García LA, Nino-Vega GA, Mora-Montes HM (2017) Fungal Strategies to evade the host immune recognition. J Fungi 3: 51
Hickman JW, Harwood CS (2008) Identification of FieQ from Pseudomonas aeruginosa as a c-di-GMP-responsive transcription factor. Mol Microbiol 69: 376–389
Hind SR, Strickler SR, Boyle PC, Dunham DM, Bao Z, O’Doherty IM, Baccile JA, Hoki JS, Viox EG, Clarke CR, et al. (2016)
Tomato receptor FLAGELLIN-SENSING 3 binds flgII-28 and activates the plant immune system. Nat Plants 2: 16128

Hirai H, Takai I, Iwano M, Nakai M, Kondo M, Takayama S, Isogai A, Che FS (2011) Glycosylation regulates specific induction of rice immune responses by Acidovorax avenae flagellin. J Biol Chem 286: 25519–25530

Hung CY, Seshan KR, Yu JJ, Schaller R, Xue J, Basur V, Gardner MJ, Cole GT (2005) A metallocprotinase of Coccidioides posadasii contributes to evasion of host detection. Infect Immun 73: 6689–6703

Ichinose Y, Taguchi F, Yamamoto M, Ohnishi-Kameyama M, Atsumi I, Iwaki M, Manabe H, Kumagai M, Nguyen QT, Nguyen CL, et al. (2013) Flagellin glycosylation is ubiquitous in a broad range of phytopathogenic bacteria. J General Plant Pathol 79: 359–365

Jashni MK, Dols IH, Iida Y, Boeren S, Beenen HG, Mehrabi R, Collemare J, De Wit PJGM (2013) Flagellin glycosylation is ubiquitous in a broad range of phytopathogenic bacteria. J General Plant Pathol 79: 359–365

Lacombe S, Rougon-Cardoso A, Sherwood E, Peeters N, Dahlbeck H, Hirai H, Takai R, Iwano M, Nakai M, Kondo M, Takayama S, Lilly WW, Stajich JE, Pukkila PJ, Wilke SK, Inoguchi N, Gathman P, Murphy C, Powolowsky J, Wu M, Butler G, Tsang A (2011) Curation of characterized glycoside hydrolases of fungal origin. Database 2011: bar020

Naumann TA, Wicklow DT, Price NP (2011) Identification of a chitinase-modifying protein from Fusarium verticillioides: truncation of a host resistance protein by a fungal metalloprotease. J Biol Chem 286: 35358–35366

Okmen B, Kemmerich B, Hilbig D, Wemhoener R, Aschenbroich J, Perrar A, Huesgen PF, Schipper K, Doehlemann G (2018) Dual function of a secreted fungal metalloprotease in Ustilago maydis. New Phytol 220: 269–261

Oliveira-Garcia E, Díezing H (2016) Attenuation of PAMP-triggered immunity in maize requires down-regulation of the key β-1,6-glucan synthesis genes KRE5 and KRE6 in birophytic hyphae of Colletotrichum graminicola. Plant J 87: 355–375

Ospina-Giraldo MD, Griffith JG, Laird EW, Mingora C (2021) The CAZyome of Phytophthora spp.: a comprehensive analysis of the gene complement coding for carbohydrate-enzyme enzymes in species of the genus Phytophthora. BMC Genomics 11: 525

Pfeilmeier S, Saur IM, Rathjen JP, Zipfel C, Malone JG (2016) High levels of cyclic-di-GMP in plant-associated Pseudomonas correlate with evasion of plant immunity. Mol Plant Pathol 17: 521–531

Poole J, Dayr CJ, von Itzstein M, Paton JC, Jennings MP (2018) Glycointeractions in bacterial pathogenesis. Nat Rev Microbiol 16: 440–452

Pradhan A, Avelar GM, Jain BM, Childers D, Pelletier C, Lacombe DE, Shekova E, Netea MG, Brown GD, Erwig L, Gow NAR, Brown AJP (2019) Non-canonical signalling mediates changes in fungal cell wall PAMPs that drive immune evasion. Nat Commun 10: 1–14

Pruitt RN, Schwessinger B, Joe A, Thomas N, Liu F, Albert M, Robinson MR, Chan LJ, Lulu DD, Chen H, et al. (2015) The rice immune receptor XA21 recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. Sci Adv 1: e1500245

Pusztaheley T (2018) Chitin and chitin-related components in plant–fungal interactions. Mycologia 9: 189–201

Pérez-de-Luque A, Tille S, Johnson I, Pascual-Pardo D, Ton J, Cameron DD (2017) The interactive effects of arbuscular mycorrhiza and plant growth-promoting rhizobacteria synergistically enhance host plant defences against pathogens. Sci Rep 7: 16409

Rapicavoli JN, Blanco-Ulate B, Muszyński A, Figueroa-Balderas R, Morales-Cruz A, Azadi P, Dobruchowska JM, Castro C, Cantu D, Roper MC (2018) Lipopolysaccharide O-antigen delays plant innate immune recognition of Xylella fastidiosa. Nat Commun 9: 399
Romero-Contreras YJ, Ramirez-Valdespino CA, Guzmán-Guzmán P, Macías-Segoviano JI, Villagómez-Castro JC, Olmedo-Monfil V (2019) Ta6 from Trichoderma atroviride is a LysM effector involved in mycoparasitism and plant association. Front Microbiol 10: 2231

Rose JK, Ham KS, Darvill AG, Albersheim P (2002) Molecular cloning and characterization of glucanase inhibitor proteins: coevolution of a counterdefense mechanism by plant pathogens. Plant Cell 14: 1329–1345

Sanz-Martín JM, Pacheco-Arjona JR, Bello-Rico V, Vargas WA, Monod M, Díaz-Minguez JM, Thon MR, Sukno SA (2016) A highly conserved metalloprotease effector enhances virulence in the maize anthermothec fungus Colletotrichum graminicola. Mol Plant Pathol 17: 1046–1062

Schellenberger R, Touchard M, Clément C, Bailleul F, Cordelier S, Crouzet J, Dorey S (2019) Apoplastic invasion patterns triggering plant immunity: plasma membrane sensing at the frontline. Mol Plant Pathol 20: 1602–1616

Segonzac C, Feike D, Gimenez-Ibanez S, Hann DR, Becker (2011) Hierarchy and roles of pathogen-associated molecular pattern-induced responses in Nicotiana benthamiana. Plant Physiol 156: 687–699

Shimada T, Park BG, Wolf AJ, Brikos C, Goodridge HS, Becker CA, Reyes CN, Miao EA, Aderem A, Götz F, et al. (2010) Staphylococcus aureus evades lysozyme-based peptidoglycan digestion that links phagocytosis, inflammasome activation, and IL-1beta secretion. Cell Host Microbe 7: 38–49

Stergiopoulos I, Van den Burg HA, Okmen B, Beenen HG, van Liere S, Kema GH, De Wit PJGM (2010) Tomato Cf resistance proteins mediate recognition of cognate homologous effectors from fungi pathogenic on dictots and monocots. Proc Natl Acad Sci USA 107: 7610–7615

Sun W, Dunning FM, Pfund C, Weingarten R, Bent AF (2006) Within-species flagellin polymorphism in Xanthomonas campestris pv campestris and its impact on elicitation of Arabidopsis flagellin. Plant Cell 18: 764–779

Taguchi F, Yamamoto M, Ohnishi-Kameyama M, Iwaki M, Yoshida M, Ishii T, Konishi T, Ichinose Y (2010) Defects in flagellin glycosylation affect the virulence of Pseudomonas syringae pv. tabaci. Microbiology 156: 72–80

Taguchi F, Takeuchi K, Katoh E, Murata K, Suzuki T, Marutani M, Kawai T, Eguchi M, Katoh S, Kaku H, et al. (2006) Identification of glycosylation genes and glycosylated amino acids of flagellin in Pseudomonas syringae pv tabaci. Cell Microbiol 8: 923–938

Takahara H, Hacquard S, Kombrink A, Hughes HB, Halder V, Robin GP, Hiruma K, Neumann U, Shinya T, Kombrink E, et al. (2016) Collettotrichum higginsianum extracellular LysM proteins play dual roles in appressorial function and suppression of chitin-triggered plant immunity. New Phytolet 211: 1322–1337

Takeuchi K, Taguchi F, Inagaki Y, Toyoda K, Shiraiishi T, Ichinose Y (2003) Flagellin glycosylation island in Pseudomonas syringae pv. glycinea and its role in host specificity. J Bacteriol 185: 6658–6665

Tang D, Wang G, Zhou JM (2017) Receptor kinases in plant-pathogen interactions: more than pattern recognition. Plant Cell 29: 618–637

Thynne E, Saur IML, Simbaqueja J, Ogilvie HA, González-Cendales Y, Mead O, Taranto A, Catanzariti AM, McDonald MC, Schwessinger B, et al. (2017) Fungal phytopathogens engage functional homologues of plant rapid alkalization factor (RALF) peptides. Mol Plant Pathol 18: 811–824

Tian M, Benedetti B, Kamoun S (2005) A Second Kazal-like protease inhibitor from Phytophthora infestans inhibits and interacts with the apoplastic pathogenesis-related protease P69B of tomato. Plant Physiol 138: 1785–1793

Tian M, Huitema E, Da Cunha L, Torto-Alabibo T, Kamoun S (2004) A Kazal-like extracellular serine protease inhibitor from Phytophthora infestans targets the tomato pathogenesis-related protease P69B. J Biol Chem 279: 26370–26377

Toruno TY, Stergiopoulos I, Coaker G (2016) Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners. Annu Rev Phytopathol 54: 419–441

Trdał L, Fernandez O, Boutot F, Hélio MC, Kellieniemi J, Diare X, Adrian M, Clément C, Zipfel C, Dorey S, Poinssot B (2014) The grapevine flagellin receptor VvFLS2 differentially recognizes flagellin-derived epitopes from the endophytic growth-promoting bacterium Burkholderia phytofirmans and plant pathogenic bacteria. New Phytolet 201: 1371–1384

Van den Burg HA, Harrison SJ, Joosten MHAJ, Vervoort J, De Wit PJGM (2006) Cladosporium fulvum Avr4 protects fungal cell walls against hydrolysis by plant chitinases accumulating during infection. Mol Plant-Microbe Interact 19: 1420–1430

Vander P, V rum KM, Domard A, Eddine EL Guedda N, Moerschbacher BM (1998) Comparison of the ability of partially N-acetylated chitosans and chitooligosaccharides to elicit resistance reactions in wheat leaves. Plant Physiol 118: 1353–1359

Vermassen A, Leroy S, Talon R, Provot C, Popowska M, Desvaux (2019) Cell wall hydrolases in bacteria: insight on the diversity of cell wall amidases, glycosidases and peptides toward peptido-glycan. Front Microbiol 10: 331

Volk H, Marton K, Flajisman M, Radisek S, Tian H, Hein I, Podlipnik C, Thomma BPHJ, Kosmeli J, Javornik B et al. (2019) Chitin-binding protein of Verticillium nonalfafae disguises fungus from plant chitinases and suppresses chitin-triggered host immunity. Mol Plant Microbe Interact 32: 1378–1390

Walls A, Tortorici M, Frenz B, Snijder J, Li W, Rey FA, DiMaio F, Bosch BJ, Veesel D (2016) Glycan shield and epitope masking of a coronavirus spike protein observed by cryo-electron microscopy. Nat Struct Mol Biol 23: 899–905

Wang F, Burgae AM, Postel S, Clark RE, Orlova A, Sundberg EJ, Kearns DB, Egelman EH (2017) A structural model of flagellar filament switching across multiple bacterial species. Nat Commun 8: 960

Wang GL, Song WY, Ruan DL, Sideris S, Ronald PC (1996) The cloned gene, Xa21, confers resistance to multiple bacterial species. Mol Plant Microbe Interact 9: 850–855

Wang S, Sun Z, Wang H, Liu L, Lu F, Yang J, Zhang M, Zhang S, Guo Z, Bent AF, Sun W (2015) Rice OsFLS2-mediated perception of bacterial flagellins is evaded by Xanthomonas oryzae pv. oryzae and oryricola. Mol Plant 8: 1024–1037

Wang S, Xing R, Wang Y, Shu H, Fu S, Paulus JK, Schuster M, Saunders DGO, Win J, Vleeshouwers V, et al. (2021) Cleavage of a pathogen apoplastic protein by plant subtilisates activates immunity. New Phytolet: doi:10.1111/nph.17710

Wawra S, Fesel P, Widmer H, Timm M, Seibel J, Kesseler L, Nostadt R, Hilbert M, Langen G, et al. (2016) Identification of flagellar epitopes from the endophytic growth-promoting bacterium Burkholderia phytofirmans. Mol Plant-Microbe Interact 29: 923–938

Yi W, Caceres-Moreno C, Jimenez-Gongora T, Wang K, Sang Y, Lozano-Duran R, Machado AP (2018) The Raisiona solanacearum csp22 peptide, but not flagellin-derived peptides, is perceived by plants from the Solanaceae family. Plant Biotechnol J 16: 1349–1362

Wolf AJ, Arruda A, Reyes CN, Kaplan AT, Shimada T, Shimada K, Arditi M, Liu G, Underhill DM (2011) Phagosomal degradation increases TLR access to bacterial ligands and enhances macrophage sensitivity to bacteria. J Immunol 187: 6002–6010

Xu Q, Wang J, Zhao J, Xu J, Sun S, Zhang H, Wu J, Tang C, Kang Z, Wang X (2020) A polysaccharide deacetylation from Puccinia striiformis f. sp. tritici is an important pathogenicity gene that suppresses plant immunity. Plant Biotechnol J 18: 1830–1842

Yamamoto M, Ohnishi-Kameyama M, Nguyen CL, Taguchi F, Chiku K, Ishii T, Ono H, Yoshida M, Ichinose Y (2011) Identification of genes involved in the glycosylation of modified viosamine of flagellins in Pseudomonas syringae by mass spectrometry. Genes 2: 788–803
Yang C, Yu Y, Huang J, Meng F, Pang J, Zhao Q, Islam MA, Xu N, Tian Y, Liu J (2019) Binding of the Magnaporthe oryzae chitinase MoChia1 by a rice tetra-TIR repeat protein allows free chitin to trigger immune responses. Plant Cell 31: 172–188

Yu X, Lund SP, Scott RA, Greenwald JW, Records AH, Nettleton D, Lindow SE, Gross DC, Beattie GA (2013) Transcriptional responses of Pseudomonas syringae to growth in epiphytic versus apoplastic leaf sites. Proc Natl Acad Sci USA 110: E425–E434

Zeng T, Rodriguez-Moreno L, Mansurkhodzaev A, Wang P, Van den Berg W, Gasciolli V, Cottaz S, Fort S, Thomma BPHJ, et al. (2020) A lysin motif effector subverts chitin-triggered immunity to facilitate arbuscular mycorrhizal symbiosis. New Phytol 225: 448–460

Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JD, Felix G, Boller T (2004) Bacterial disease resistance in Arabidopsis through flagellin perception. Nature 428: 764–767

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell 125: 749–760