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Disease induction by human microbial pathogens in plant-model systems: potential, problems and prospects

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Relatively simple eukaryotic model organisms such as the genetic model weed plant *Arabidopsis thaliana* possess an innate immune system that shares important similarities with its mammalian counterpart. In fact, some human pathogens infect *Arabidopsis* and cause overt disease with human symptomology. In such cases, decisive elements of the plant's immune system are likely to be targeted by the same microbial factors that are necessary for causing disease in humans. These similarities can be exploited to identify elementary microbial pathogenicity factors and their corresponding targets in a green host. This circumvents important cost aspects that often frustrate studies in humans or animal models and, in addition, results in facile ethical clearance.

Introduction

Animals and plants belong to different kingdoms and display an obviously different structural organization at the subcellular, cellular and tissue level [1]. Both types of organisms have numerous potential microbial pathogens, but the overlap in microbial pathogens between animals and plants has attracted little scientific attention. However, some pathogens, sometimes referred to as cross-kingdom pathogens, can infect organisms from different kingdoms and use them successfully as hosts. Several examples of mammalian pathogens that have been reported as plant pathogens, and vice versa, have been documented. In addition, it is increasingly being recognized that fresh produce can harbor human pathogens of which *Bacillus cereus*, *Campylobacter* spp., *Clostridium botulinum*, *Escherichia coli*, *Listeria monocytogenes*, *Shigella* spp., *Salmonella* spp. and *Yersinia enterocolitica* are the most notorious [2]. It is clear that crops can become contaminated easily with several of these pathogens. During growth and cultivation in soils that are contaminated with pathogen-containing animal manure, many of these pathogens find an opportunity to shift host. Such indirect zoonotic transfer can be much more frequent than anticipated. Interestingly, in several cases, it has been demonstrated that these pathogens not only occur on the surfaces of these vegetables but also inside the plant tissues [3]. Furthermore, in some cases plants have been used as heterologous hosts for specific mammalian pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* [4]. Although these pathogens seem to share the basic abilities to infect plants under specific conditions, they have rarely or never been reported to cause plant infections in nature.

The model plant *Arabidopsis thaliana* is especially useful as a heterologous host for several mammalian pathogens (Table 1). The *Arabidopsis* models offer several advantages over animal models, including ease of rearing, short generation time, a completely sequenced genome (http://www.arabidopsis.org), ease and versatility to be used in molecular studies [5,6], the availability of mutants for nearly every gene in the genome [7] and, perhaps most important, exemption from costs and ethical issues associated with using mammalian laboratory test organisms. Using *Arabidopsis*, it has been possible to identify microbial pathogenicity factors that contribute to disease development, not only in the plant host but also in the mammalian host (Table 1). Furthermore, it has been documented that the same regulatory mechanisms leading to attenuated virulence in humans often play decisive parts in diminishing the deleterious effect during plant infections.

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TABLE 1

Selected human bacterial pathogens of which pathogenicity has been successfully studied using an *Arabidopsis* plant-model system

Species	Pathogenicity factors	References
Pseudomonas aeruginosa	Exotoxin A, global activator (*gac)A*, phospholipase C	[31,34,51,79]
Staphylococcus aureus	α-Toxin, staphylococcal accessory gene regulator (sar)A, accessory gene regulator (agr) – quorum sensing	[35]
Enterococcus faecalis	Serine protease, *Enterococcus faecalis* regulator (*fsr)* – quorum sensing	[80]

Conservation of innate immunity in plants and animals

The innate immune systems of plants and vertebrates share important similarities and function to ward off microbial pathogens in similar ways [8,9]. These similarities include molecular structures that are as diverse as the receptors involved in pathogen recognition, mitogen-associated protein-kinase-based downstream signaling pathways, use of reactive oxygen species in direct defense (i.e. respiratory burst) and the production of antimicrobial peptides. One class of antimicrobial peptides that is found to be conserved across kingdom barriers is the defensins, and members have been identified in plants, fungi, invertebrate animals and vertebrates [10,11]. Such peptides are frequently induced upon infections in mammalian hosts. Neutral nasal carriage of the bacterial species *S. aureus* by a human host leads to local induction of defensin synthesis, leading to an apparent low-grade state of infection without complication [12]. Similarly, plant defensins seem to be an important component of basal host defense in interactions with compatible microbial pathogens [10,13].

A comparison of ~300 human disease-associated genes shows that almost 80% have an ortholog in the insect *Drosophila melanogaster*, ~70% have an ortholog in the nematode *Caenorhabditis elegans* [14] and *Arabidopsis* still possesses orthologs for ~60% of these human disease-associated genes (http://mipsgsfde/proj/thal/db/tables/tables_comp_framehtml). This suggests that the molecular processes required for host defense share a high degree of similarity, and that the ability of a pathogen to overcome a particular defense mechanism in one host could have profound implications for the capacity of the pathogen to overcome similar mechanisms in other hosts. To date, no system or process comparable to the vertebrate complement system has been found in plants or invertebrates.

Pathogen perception displays important similarities between vertebrates and plants

Innate immunity is activated by specific recognition of microbial invaders based on pattern-recognition receptors (PRRs), which respond to microbial-associated molecular patterns (MAMPs) and pathogen-effector molecules. MAMPs include Gram-negative bacterial lipopolysaccharide and peptidoglycan of Gram-positive bacteria, bacterial flagellin and nucleic acids [8,15] that are not produced by the host itself and can, therefore, be used to distinguish self from non-self [15,16]. Effectors could be released by intercellular secretion or injected into host cells by bacterial type III secretion systems. Recently, it has been found that effector molecules that are secreted intercellularly by mammalian and plant pathogens can similarly be targeted into host cells using a specific host-targeting (HT) motif [17].

The PRRs include so-called Toll-like receptors (TLRs), a family of conserved transmembrane proteins composed of an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic TIR (Toll/interleukin-1 receptor) domain (i.e. homologous to the cytoplasmic domain of Toll and the human interleukin (IL)-1 receptor [18,19]). In plants, PRRs containing extracellular LRRs comprise receptor-like proteins (RLPs) and receptor-like kinases (RLKs) [20,21]. In addition to these extracellular receptors, intracellular PRRs have also been identified. These include the mammalian NLR (NACHT-LRR) and plant nucleotide-binding site (NBS)-LRR proteins [22,23].

Once a pathogen has been recognized, acute host responses often depend on kinase activity mediated by a conserved family of serine–threonine kinases. The kinase domain that occurs in the cytoplasmic component of plant RLKs bears a resemblance to IL-1 receptor-associated kinase (IRAK) from mammals and Pelle kinases from *Drosophila* [23]. Downstream of these serine–threonine kinases, mitogen-activated protein (MAP) kinase cascades are activated, calcium fluxes occur, reactive oxygen is produced and transcription factors that activate expression of defense-response effectors such as antimicrobial peptides and proteins [8,9] and the induction of programmed cell death (PCD) are expressed. The outcome of host–pathogen interactions often depends on occurrence of host-cell death.

Conserved microbial effectors operational in animals and plants

Typically, disease establishment depends on the modulation (i.e. by active suppression or promotion) of cell death in susceptible hosts. Such modulations are usually achieved by diverse microbial effectors that target processes conserved in animals and plants. For instance, the involvement of protein ubiquitination in relation to PCD has been demonstrated in many organisms [24]. The phytopathogenic bacterium *Pseudomonas syringae,* which has not been identified as an infectious agent in humans so far, injects the effector protein avirulence protein B from *P. syringae* pv. tomato (AvrPtoB) directly into plant cells. AvrPtoB displays ubiquitin ligase activity and inhibits plant PCD initiated by the plant's disease-resistance proteins [25]. Attenuation of plant-cell death is important because successful leaf colonization and bacterial proliferation depend on delayed host-cell death. Remarkably, AvrPtoB can also suppress PCD in baker's yeast (i.e. *Saccharomyces cerevisiae*), indicating that AvrPtoB acts on PCD components that seem to be conserved across different kingdoms [26]. Because of such conservation, plant models can be used to investigate and characterize aspects of microbial pathogenicity towards vertebrates.

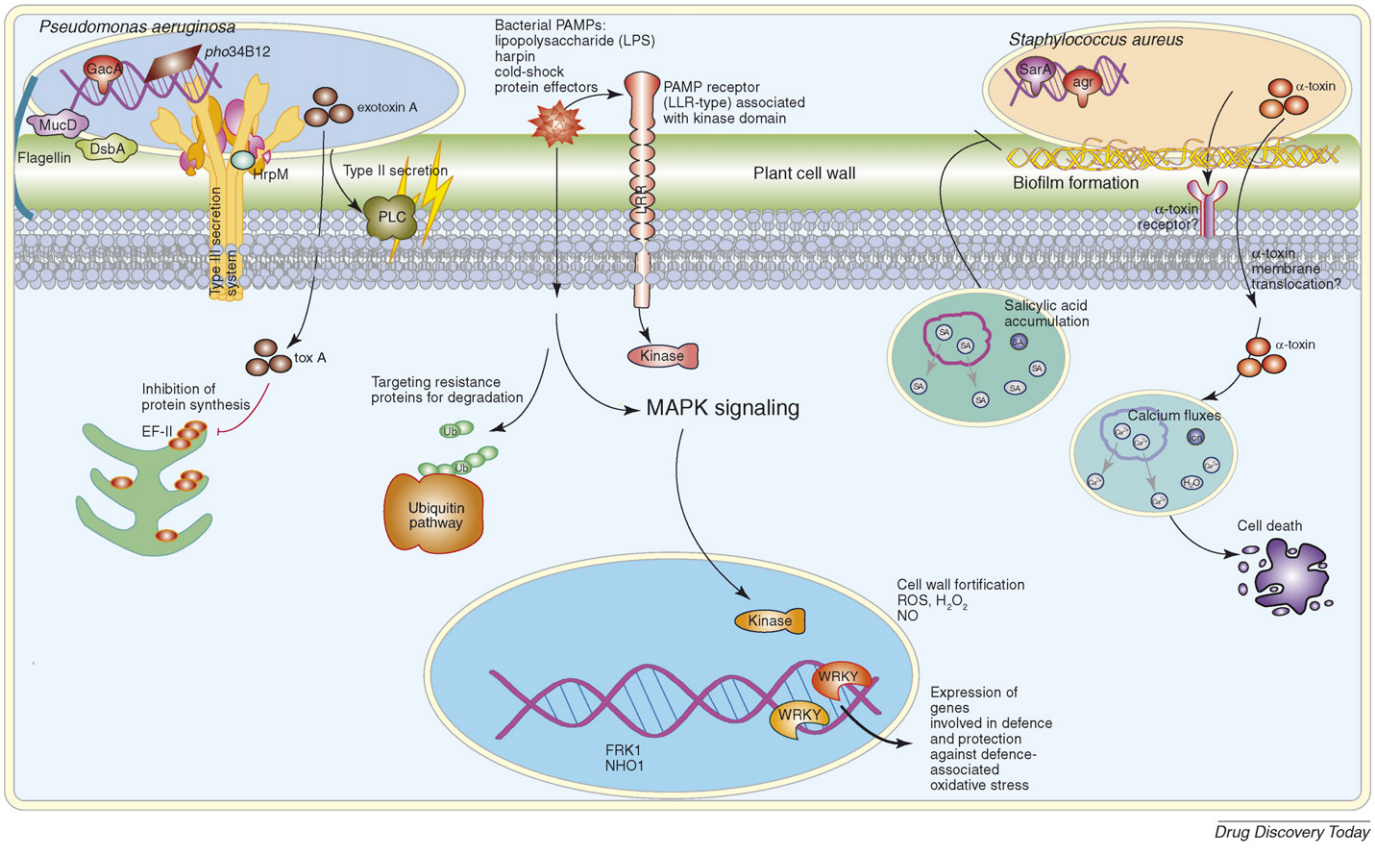

FIGURE 1

Bacterial pathogenicity factors and associated host responses that have been identified in human and *Arabidopsis* plant hosts. The innate immune systems of plants and vertebrates share important similarities and function to ward off microbial pathogens in similar ways. The figure shows host signaling cascades induced by *Pseudomonas aeruginosa* and *Staphylococcus aureus* as examples.

Examples of successful plant-model system usage

Based on the similarities between plants and animals, several research groups have evaluated and discussed the use of plant models to study microbial pathogenicity [4,8,27–31]. In this review, we discuss a few examples (see also Figure 1) and suggest how models can be used to further explore microbial pathogenicity factors, their mode of action and their corresponding human targets.

The effectivity of salicylic acid to suppress bacterial pathogenicity

400 years BC, Hippocrates prescribed the use of *Salix alba* (i.e. willow) bark to patients to relieve pain and fever. In the 20th century the active ingredient was identified as the phenolic metabolite salicylic acid (SA). It was found that SA acts as a plant hormone that triggers systemic immunity [32,33]. In addition, using the *Arabidopsis* model system, it has been demonstrated that SA counteracts the virulence of the human pathogen *P. aeruginosa* by repressing attachment, biofilm formation and the production of virulence factors [34]. SA-treated bacteria accumulated to densities similar to those attained by untreated bacteria, but they were less capable of killing *C. elegans* nematodes, supporting the hypothesis that SA directly influences the expression of virulence factors [34]. Similar results were obtained in trials in which *Arabidopsis* was used as a host for the human pathogenic bacterium *S. aureus* [4,35]. Importantly, intravenous aspirin [acetylsalicylic acid (ASA)] was shown to result in a reduction of *S. aureus* densities on the endocardial surface in a rabbit model of invasive endocarditis. This effect could be attributed to ASA retention of the inhibitory properties of SA, its precursor molecule. In this interaction, pretreatment of bacteria with SA considerably reduced attachment to the cardiac epithelium [36]. Interestingly, ASA also seems to be effective in the elimination of *S. aureus* colonization [37].

SA is a successful example of a plant metabolite that is converted into a drug. However, in many ancient or isolated cultures, traditional healers employ remedies made from plants to combat disease, and therefore medicinal plants play an important part in the health of millions of people. Such medicinal activities might be attributed to the presence of specific compounds in those plant species. To date, >50,000 natural products have been identified, of which many play parts in plant defense against herbivores and pathogens [38]. Single plant species, such as *Arabidopsis*, can make thousands of secondary metabolites (i.e. low-molecular-weight compounds) as by-products of normal metabolism, many of which have anti-

microbial activity [39,40]. One of the better-studied secondary metabolites is the indole phytoalexin from *Arabidopsis* – called camalexin. The biological importance of camalexin had been uncovered in a screen for plants with reduced resistance to the fungal pathogen *Alternaria* [13,41], but this compound is also found to be toxic to other pathogens [42]. Similarly, secondary metabolites called glucosinolates are found to display antimicrobial activity [43,44]. Given the wealth of metabolites that are produced by a single plant species, and the observation that many of these can display antimicrobial activity, plant studies aimed at screening for such activity against different pathogens might result in the identification of bioactive compounds. Such compounds can be used directly for treatment if they can be purified on a large scale [45], or can form the basis for the development of novel drugs. However, chemical synthesis of these compounds or derivatives with similar activities might also be considered, although the biosynthetic pathways that are responsible for the production of such metabolites tend to be complicated and difficult to unravel [46].

Identification of pathogenicity factors of the bacterial pathogen Pseudomonas aeruginosa

P. aeruginosa is a Gram-negative saprotrophic bacterial species that is ubiquitously present in the environment. It is a major cause of wound infections, sepsis in burned and immunodeficient patients, and lung colonization and infection in humans suffering from cystic fibrosis (CF). By contrast, *P. aeruginosa* rarely causes infections in immunocompetent patients. However, all *P. aeruginosa* isolates essentially possess the basic machinery to cause human infections regardless of the habitat from which they are isolated [47]. *In vivo* mutation rates are elevated; during *in vivo* (i.e. pathogenic) growth, isolates showed positive, or diversifying, selection across the whole genome, especially in those regions involved in pathogenicity [48]. It has been demonstrated that *P. aeruginosa* isolates with elevated mutation rates can be isolated from lung tissue from approximately one-third of persistently infected CF patients, but scarcely at all from patients suffering from opportunistic *P. aeruginosa* infections [49]. *In vivo* adaptation towards a host is an important feature of pathogens and can be stress-related, for example in reaction to host defense responses [50]. In several laboratory studies, clinical isolates of *P. aeruginosa* were shown to infect *Arabidopsis*, tobacco and lettuce successfully [30,31]. The observation that different *Arabidopsis* ecotypes display differential degrees of resistance to different *P. aeruginosa* isolates has been interpreted to suggest that this microorganism might infect *Arabidopsis* under natural conditions [51]. Microbial adaptation and subsequent conservation of the 'optimal fit' has been observed in humans. For instance, the maturation of the colonic flora during early life shows that close matching of microbe and host is a feature that ultimately leads to the establishment of a personalized, fingerprint-type intestinal microbiota composition [52].

Many bacterial cell-associated and secreted factors that play a part in *P. aeruginosa* virulence in plant and animal hosts have been identified. These include flagella and type IV pili, type III secretion systems, lipopolysaccharide, protease, endo- and exo-toxin, and the mucous exopolysaccharide alginate. The production of these virulence factors is regulated by environmental stimuli and by quorum-sensing cascades [31]. Remarkably, several bacterial mutants have been identified that display reduced virulence in plant and animal hosts. Bacterial mutants at the exotoxin A gene (i.e. a protein synthesis inhibitor), the phospholipase C gene, which is involved in phospholipid degradation, and the *gacA* gene, which is a transcriptional activator of effector genes, were found to display pathogenicity that was lower than wild-type levels in mice and *Arabidopsis* [30] (Figure 1). Similar studies with these and additional mutants in other plant and vertebrate hosts have shown that several factors promote virulence on multiple hosts, and that a subset of the existing virulence factors is required for full pathogenicity in all hosts [31].

Alternaria *toxins modulating host-cell necrosis and programmed cell death*

Alternaria pathogens infect plant and animal hosts and cause disease by producing diverse toxic metabolites [53]. The most intensively studied *Alternaria* toxin is *Alternaria alternata* f. sp. *lycopersici* (AAL)-toxin, produced by the tomato pathogen *Alternaria alternata* f.sp *lycopersici*. AAL-toxin is highly toxic to plants and animals including humans [54]. AAL-toxin is an aminopentol ester that structurally resembles the mycotoxin fumonisin B1. This latter toxin was originally identified in *Fusarium verticillioides* (formerly called *Fusarium moniliforme*) cultures, but is also produced by AAL-toxin-producing *A. alternata* isolates [55]. Interestingly, fumonisin B1 is also selectively toxic to AAL-toxin-sensitive plant genotypes [56]. Both toxins are potent inhibitors of plant sphingolipid and ceramide biosynthesis [54,57,58]. The type of cell death induced by AAL-toxin is reminiscent of apoptosis. Indeed, transgenic tomato plants expressing a viral anti-apoptotic gene were protected against AAL-toxin-induced cell death and pathogen infection [59]. Modulation of the host apoptotic suicide program is a common strategy of microbial pathogens. *S. aureus* can enhance but can also inhibit apoptosis of vertebrate host cells during infection, depending on the bacterial (i.e. extracellular) proteins secreted and the nature of the host cell [60,61]. Investigating the regulation and execution of plant and mammalian apoptosis might, therefore, reveal how pathogens modulate apoptosis, and might result in the identification of important drug targets. In a microarray study of AAL-toxin-induced apoptosis in *Arabidopsis*, oxidative burst and other defence-related pathways, PCD-related proteases and transcription factors were among the first plant responses and upregulated genes [62] showing that genome-wide studies and omics approaches are feasible in *Arabidopsis*. Considering the ongoing *Arabidopsis* protein annotation and functional analyses (http://www.arabidopsis.org), target discovery can only be expected to accelerate.

Shortcomings and strengths of the plant models

In this review, previous sections have addressed the successes of translating human infections to 'artificial' plant hosts. However, the research questions raised in clinical infectiology still, by far, outnumber the answers obtained to date by cross-kingdom infection studies. In the pathogenesis of many infections, carriage before invasion is important. In the case of *S. aureus*, for instance, knowledge of the functional determinants defining this transition is largely missing. When using a plant model, one would need to establish the similarities that are mandatory to mimick such a process. An important question would be whether the same

ligands are available for plant colonization. *S. aureus* has receptors for a wide variety of human cell-surface compounds. These include haptoglobin, hemoglobin, cytokeratins, fibronectin, fibrinogen, vitronectin-transferring lactoferrin and collagen, among others. So far, none of these proteins have been functionally identified in plant tissues, although some of these ligands have homologues in plants. Hemoglobins [63], fibronectin-like protein [64], vitronectin-related protein [65] and glycine-rich proteins that resemble collagen [66] have been detected in plants or plant genomes. Many of these receptors also play a part in the infection of humans, but additional virulence factors such as superantigens, toxins and immunomodulatory compounds steer the infectious process [66–69]. In addition, *S. aureus* secretes a wide variety of proteins that could act remotely from the infection or the colonization site [70]. Whether such compounds will be able to circulate adequately in plant hosts is doubtful. It remains to be seen whether the plant host will ultimately show compatibility with such mandatory requirements, not only for *S. aureus* but also for other human microbial pathogens.

As noted, being able to use plant models to study pathogenicity of human pathogens relies on the microbial capacity for cross-kingdom pathogenicity. So far, cross-kingdom pathogenicity has been attributed to few microbial taxa [27], but this group contains highly important pathogens such as the bacteria *P. aeruginosa*, *C. botulinum* and *L. monocytogenes*, and fungal *Alternaria* species. The virulence factors that cause host cellular damage (i.e. disease) are often proteases or pore-forming (hemo)lysins that interfere with membrane integrity and toxins that target fundamental and highly conserved cellular mechanisms. Using plant models, the modes of action of such microbial factors and their corresponding host targets can be investigated using thousands of genetically similar hosts (e.g. mutant libraries) in a cost-effective way with no need to apply for ethical clearance. The existence of *Arabidopsis* mutants that respond with accelerated cell death to microbial virulence factors has already been demonstrated; these mutants are publicly available and amenable to laboratory experimentation involving bacterial and fungal pathogens [71–73].

Specificity of microbial virulence factors in cross-kingdom pathogens can be such that different effectors are required for establishing disease in plants or animals. The genes encoding such factors can be organized in specific genomic regions, so-called pathogenicity islands, which can be horizontally transmitted between strains or even species (e.g. possibly by bacteriophage activity) [74]. Horizontal transfer of genes from the animal pathogen *P. aeruginosa* (PA)O1 and subsequent further diversification have both been suggested to have resulted in the current genetic makeup of a pathogenic *P. syringae* isolate [75]. Specificity of virulence factors for either plant or animal hosts potentially hampers *in planta* screens for factors that are operational in animals. However, these problems are likely to arise with every heterologous pathogen model-infection system. For example, it has been shown that virulence factors of the human pathogen *S. aureus* display differential activity in a mouse model [67]. Moreover, virulence gene products can even demonstrate specificity to such an extent that they confer the ability to survive in one specific differentiated cell type, for example immune cells [76]. Nevertheless, despite specificity for a certain mammalian cell type, cross-kingdom pathogenicity can be established based on the existence of reminiscent cell types in an alternative host [77]. Overall, we believe that plant models will provide unprecedented possibilities to study microbial pathogenicity, making them valuable tools when studying human pathogens.

Concluding remarks and future perspectives

Currently, use of simple eukaryotic models for the identification of innate immune responses and their targets, usually coined pathogenicity factors, is widespread in clinical microbiology and infectiology, and can, to a certain degree, give insight into the genetically less-tractable human host biology [28]. Experimental studies have shown that the model plant *Arabidopsis* is useful for exploring microbial pathogenicity factors that are also of clinical relevance to humans. Given the ease by which plants can be used in large-scale and high-throughput screens, plants are attractive model hosts in inoculation studies involving bacterial or fungal pathogens. *Arabidopsis* has been used in several successful trials aimed at identifying pathogen-effector molecules, and is amenable to various large-scale omics studies. Plant models are valuable alternatives to mammalian hosts because of their versatility and ease of use – probably for direct clinical studies as well.

Plant models hold screening potential for diverse microbial virulence factors. *Arabidopsis* plant models are not only cost-effective but also provide the opportunity of screening a diverse array of homozygous mutants, and do not require ethical clearance. Interesting classes of mutants display accelerated cell death or are more tolerant to cell-death signals, or display other features associated with pathogen infection, tolerance and susceptibility. These categories of mutants could eventually reveal receptors targeted by microbial virulence factors, or could result in identification of low-molecular-weight metabolites that either interfere with virulence factors or are directly toxic to microbes. *Arabidopsis* model systems are also amenable to studies to co-express or silence receptors and potential corresponding virulence factors.

To date, the major contribution of plant science to drug development has been limited to the discovery of bioactive compounds that can be commercialized to treat infections or other diseases. In addition to SA, the production of the anticancer agent taxol by *Taxus* cell cultures is another example of successful industrialization for commercial application. In particular, plant secondary metabolites are discovered as a rich source for antimicrobials and considerable efforts are put into studies for the commercial production of such compounds [45,78]. In our view, screening for pathogen-virulence targets and using plants as a model for human diseases is the next step in the contribution of plant science to drug development. Although results of the successful use of plant models for human diseases have not been publicly announced yet, it is anticipated that plant screens are going to be deployed in the drug discovery industry or in life-science companies. The use of plant models should be considered as part of contemporary research aimed at revealing key processes involved in establishing host–pathogen interactions.

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References

1 Whittaker, R.H. (1959) On the broad classification of organisms. *Q. Rev. Biol.* 34, 210–226
2 Beuchat, L.R. (2002) Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infect.* 4, 413–423
3 Brandl, M.T. (2006) Fitness of human enteric pathogens on plants and implications for food safety. *Annu. Rev. Phytopathol.* 44, 367–392
4 Prithiviraj, B. *et al.* (2005) Plant models for animal pathogenesis. *Cell. Microbiol.* 7, 315–324
5 Pang, P.P. and Meyerowitz, E.M. (1987) *Arabidopsis thaliana*: a model system for plant molecular biology. *Nat. Rev. Biotechnol.* 5, 1177–1181
6 Meinke, D.W. *et al.* (1998) *Arabidopsis thaliana*: a model plant for genome analysis. *Science* 662, 679–682
7 Alonso, J.M. *et al.* (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* 301, 653–657
8 Abramovitch, R.B. *et al.* (2006) Bacterial elicitation and evasion of plant innate immunity. *Nat. Rev. Mol. Cell Biol.* 7, 601–611
9 Ausubel, F.M. (2005) Are innate immune signaling pathways in plants and animals conserved? *Nat. Immunol.* 6, 973–979
10 Thomma, B.P.H.J. *et al.* (2002) Plant defensins. *Planta* 216, 193–202
11 Mygind, P.H. *et al.* (2005) Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. *Nature* 437, 975–980
12 Cole, A.M. *et al.* (2001) Determinants of *Staphylococcus aureus* nasal carriage. *Clin. Diagn. Lab. Immunol.* 8, 1064–1069
13 Thomma, B.P.H.J. *et al.* (1998) Separate jasmonate-dependent and salicylate-dependent defense response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15107–15111
14 Rubin, G.M. *et al.* (2000) Comparative genomics of the eukaryotes. *Science* 287, 2204–2215
15 Nürnberger, T. *et al.* (2004) Innate immunity in plants and animals: striking similarities and obvious differences. *Immunol. Rev.* 198, 249–266
16 Medzhitov, R. and Janeway, C.A. (2002) Decoding the patterns of self and nonself by the innate immune system. *Science* 296, 298–300
17 Bhattacharjee, S. *et al.* (2006) The malarial host-targeting signal is conserved in the Irish potato famine pathogen. *PLoS Pathog.* 2, 453–465
18 Lemaitre, B. *et al.* (1997) *Drosophila* host defence: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc. Natl. Acad. Sci. U. S. A.* 94, 14614–14619
19 Medzhitov, R. *et al.* (1997) A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388, 394–397
20 Song, W.-Y. *et al.* (1995) A receptor kinase-like protein encoded by the rice disease resistance geneXa27. *Science* 270, 1804–1806
21 Jones, D.A. *et al.* (1994) Isolation of the tomato Cf-9 gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science* 266, 789–793
22 Martinon, F. and Tschopp, J. (2005) NLRs join TLRs as innate sensors of pathogens. *Trends Immunol.* 26, 447–454
23 Shiu, S.-H. and Bleecker, A.B. (2001) Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. *Proc. Natl. Acad. Sci. U. S. A.* 98, 10763–10768
24 Hershko, A. and Ciechanover, A. (1998) The ubiquitin system. *Annu. Rev. Biochem.* 67, 425–479
25 Janjusevic, R. *et al.* (2006) A bacterial inhibitor of host programmed cell death defences is an E3 ubiquitin ligase. *Science* 311, 222–226
26 Abramovitch, R.B. *et al.* (2003) *Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. *EMBO J.* 22, 60–69
27 Cao, H. *et al.* (2001) Common mechanisms for pathogens of plants and animals. *Annu. Rev. Phytopathol.* 39, 259–284
28 Casadevall, A. (2005) Host as the variable: model hosts approach the immunological asymptote. *Infect. Immun.* 73, 3829–3832
29 Plotnikova, J.M. *et al.* (2001) Pathogenesis of the human opportunistic pathogen *Pseudomonas aeruginosa* PA14 in *Arabidopsis*. *Plant Physiol.* 124, 1766–1774
30 Rahme, L.G. *et al.* (1995) Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 268, 1899–1902
31 Rahme, L.G. *et al.* (2000) Plants and animals share functionally common bacterial virulence factors. *Proc. Natl. Acad. Sci. U. S. A.* 97, 8815–8821
32 Delaney, T.P. *et al.* (1994) A central role of salicylic acid in plant disease resistance. *Science* 266, 1247–1250
33 Thomma, B.P.H.J. *et al.* (2001) The complexity of disease signaling in *Arabidopsis*. *Curr. Opin. Immunol.* 13, 63–68
34 Prithiviraj, B. *et al.* (2005) Down regulation of virulence factors of *Pseudomonas aeruginosa* by salicylic acid attenuates its virulence on *Arabidopsis thaliana* and *Caenorhabditis elegans*. *Infect. Immun.* 73, 5319–5328
35 Prithiviraj, B. *et al.* (2005) *Staphylococcus aureus* pathogenicity on *Arabidopsis thaliana* is mediated either by a direct effect of salicylic acid on the pathogen or by SA-dependent, NPR1-independent host responses. *Plant J.* 42, 417–432
36 Kupferwasser, L.I. *et al.* (1999) Acetylsalicylic acid reduces vegetation bacterial density, hematogenous bacterial dissemination, and frequency of embolic events in experimental *Staphylococcus aureus* endocarditis through antiplatelet and antibacterial effects. *Circulation* 99, 2791–2797
37 Karabay, O. *et al.* (2006) A new effect of acetylsalicylic acid? Significantly lower prevalence of nasal carriage of *Staphylococcus aureus* among patients receiving orally administered acetylsalicylic acid. *Infect. Control Hosp. Epidemiol.* 27, 318–319
38 Osbourn, A.E. *et al.* (2003) Dissecting plant secondary metabolism – constitutive chemical defences in cereals. *New Phytol.* 159, 101–108
39 Dixon, R.A. (2001) Natural products and plant disease resistance. *Nature* 411, 843–847
40 Hahlbrock, K. *et al.* (2003) Non-self recognition, transcriptional reprogramming, and secondary metabolite accumulation during plant/pathogen interactions. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14569–14576
41 Thomma, B.P.H.J. *et al.* (1999) Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. *Plant J.* 19, 163–171
42 Kliebenstein, D.J. *et al.* (2005) Secondary metabolites influence *Arabidopsis/Botrytis* interactions: variation in host production and pathogen sensitivity. *Plant J.* 44, 25–36
43 Tierens, K.F.M-J. *et al.* (2001) Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of *Arabidopsis* to microbial pathogens. *Plant Physiol.* 125, 1688–1699
44 Brader, G. *et al.* (2006) Altering glucosinolate profiles modulates disease resistance in plants. *Plant J.* 46, 758–767
45 Zhao, J. *et al.* (2005) Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.* 23, 283–333
46 Schuhegger, R. *et al.* (2006) CYP71B15 (PAD3) catalyzes the final step in camalexin biosynthesis. *Plant Physiol.* 141, 1248–1254
47 Wolfgang, M.C. *et al.* (2003) Conservation of genome content and virulence determinants among clinical and environmental isolates of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8484–8489
48 Smith, E.E. *et al.* (2006) Genetic adaptation of *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc. Natl. Acad. Sci. U. S. A.* 103, 8487–8492
49 Oliver, A. *et al.* (2000) High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 288, 1251–1254
50 Brown, N.F. *et al.* (2006) Crossing the line: selection and evolution of virulence traits. *Plos Pathog.* 2, e42
51 Rahme, L.G. *et al.* (1997) Use of model plant hosts to identify *Pseudomonas aeruginosa* virulence factors. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13245–13250
52 Gill, S.R. *et al.* (2006) Metagenomic analysis of the human distal gut microbiome. *Science* 312, 1355–1359
53 Thomma, B.P.H.J. (2003) *Alternaria* spp: from general saprophyte to specific parasite. *Mol. Plant Pathol.* 4, 225–236
54 Abbas, H.K. *et al.* (1994) Fumonisin- and AAL-toxin-induced disruption of sphingolipid metabolism with accumulation of free sphingoid bases. *Plant Physiol.* 106, 1085–1093
55 Chen, J. *et al.* (1992) Production of the mycotoxin fumonisin B1 by *Alternaria alternata* f.sp. *lycopersici*. *Appl. Environ. Microbiol.* 58, 3928–3931
56 Gilchrist, D.G. *et al.* (1992) Genetic and physiological response to fumonisins and AAL-toxin by intact tissue of a higher plant. *Mycopathologia* 117, 57–64
57 Wang, H. *et al.* (1996) Apoptosis: a functional paradigm for programmed plant cell death induced by a hostselective phytotoxin and invoked during development. *Plant Cell* 8, 375–391

58 Wang, W. *et al.* (1996) Fumonisins and *Alternaria alternata lycopersici* toxins: sphinganine analog mycotoxins induce apoptosis in monkey kidney cells. *Proc. Natl. Acad. Sci. U. S. A.* 93, 3461–3465
59 Lincoln, J.E. *et al.* (2002) Expression of the antiapoptotic baculovirus p35 gene in tomato blocks programmed cell death and provides broadspectrum resistance to disease. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15217–15221
60 Bratton, D.L. *et al.* (1999) Staphylococcal toxic shock syndrome toxin-1 inhibits monocyte apoptosis. *J. Allergy Clin. Immunol.* 103, 895–900
61 Mempel, M. *et al.* (2002) Invasion of human keratinocytes by *Staphylococcus aureus* and intracellular bacterial persistence represent haemolysin-independent virulence mechanisms that are followed by features of necrotic and apoptotic keratinocyte cell death. *Br. J. Dermatol.* 146, 943–951
62 Gechev, T.S. *et al.* (2004) An extensive microarray analysis of AAL-toxin-induced cell death in *Arabidopsis thaliana* brings new insights into the complexity of programmed cell death in plants. *Cell. Mol. Life Sci.* 61, 1185–1197
63 Kundu, S. *et al.* (2003) Plants, humans and hemoglobins. *Trends Plant Sci.* 8, 387–393
64 Cossard, E. *et al.* (2005) Comparative adherence to human A549 cells, plant fibronectin-like protein, and polystyrene surfaces of four *Pseudomonas fluorescens* strains from different ecological origin. *Can. J. Microbiol.* 51, 811–815
65 Zhu, J.K. *et al.* (1994) A higher plant extracellular vitronectin-like adhesion protein is related to the translational elongation factor-1 alpha. *Plant Cell* 6, 393–404
66 Ringli, C. *et al.* (2001) Glycine-rich proteins as structural components of plant cell walls. *Cell. Mol. Life Sci.* 58, 1430–1441
67 Foster, T.J. (2005) Immune evasion by staphylococci. *Nat. Rev. Microbiol.* 3, 948–958
68 Murray, R.J. (2005) Recognition and management of *Staphylococcus aureus* toxin-mediated disease. *Intern. Med. J.* 35 (suppl.), S106–S119
69 Rooijakkers, S.H. *et al.* (2005) Staphylococcal innate immune evasion. *Trends Microbiol.* 13, 596–601
70 Sibbald, M.J. *et al.* (2006) Mapping the pathways to staphylococcal pathogenesis by comparative secretomics. *Microbiol. Mol. Biol. Rev.* 70, 755–788
71 Greenberg, J.T. and Ausubel, F.M. (1993) *Arabidopsis* mutants compromised for the control of cellular damage during pathogenesis and aging. *Plant J.* 4, 327–341
72 Asai, T. *et al.* (2000) Fumonisin B1–induced cell death in *Arabidopsis* protoplasts requires jasmonate-, ethylene-, and salicylate-dependent signaling pathways. *Plant Cell* 12, 1823–1836
73 van Baarlen, P. *et al.* Histochemical and genetic analysis of host and non-host interactions of *Arabidopsis* with three *Botrytis* species: an important role for cell death control. *Mol. Plant Pathol.* (in press)
74 He, J. *et al.* (2004) The broad host range pathogen *Pseudomonas aeruginosa* strain PA14 carries two pathogenicity islands harboring plant and animal virulence genes. *Proc. Natl. Acad. Sci. U. S. A.* 101, 2530–2535
75 Joardar, V. *et al.* (2005) Lineage-specific regions in *Pseudomonas syringae* pv tomato DC3000. *Mol. Plant Pathol.* 6, 53–64
76 Ochman, H. *et al.* (1996) Identification of a pathogenicity island required for *Salmonella* survival in host cells. *Proc. Natl. Acad. Sci. U. S. A.* 93, 7800–7804
77 Steenbergen, J.N. *et al.* (2001) *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proc. Natl. Acad. Sci. U. S. A.* 98, 15245–15250
78 Zhao, J. *et al.* (2005) Oxidative stress in plant cell culture: a role in production of β-thujaplicin by *Cupressus lusitanica* suspension culture. *Biotechnol. Bioeng.* 90, 621–631
79 Mahajan-Miklos, S. *et al.* (2000) Elucidating the molecular mechanisms of bacterial virulence using non-mammalian hosts. *Mol. Microbiol.* 37, 981–988
80 Jha, A.K. *et al.* (2005) *Enterococcus faecalis* mammalian virulence-related factors exhibit potent pathogenicity in the *Arabidopsis thaliana* plant model. *Infect. Immun.* 73, 464–475