Molecular Plant • Volume 7 • Number 4 • Pages 657–674 • April 2014 RESEARCH ARTICLE

Salmonella enterica Flagellin Is Recognized via FLS2 and Activates PAMP-Triggered Immunity in *Arabidopsis thaliana*

Ana Victoria Garcia[a,1], Amélie Charrier[a], Adam Schikora[b], Jean Bigeard[a], Stephanie Pateyron[c], Marie-Ludivine de Tauzia-Moreau[a], Alexandre Evrard[a], Axel Mithöfer[d], Marie Laure Martin-Magniette[a,e,f], Isabelle Virlogeux-Payant[g], and **Heribert Hirt[a]**

a Unité de Recherche en Génomique Végétale (URGV), UMR INRA/CNRS/Université d'Evry Val d'Essonne, 91057 Evry, France
b Institute for Plant Pathology and Applied Zoology, Research Centre for BioSystems, Land Use and Nutrition, JL University Giessen, Heinrich-Buff-Ring 26–32, 35392 Giessen, Germany
c Transcriptomic Platform, Unité de Recherche en Génomique Végétale (URGV), 91057 Evry, France
d Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany
e INRA, UMR 518 MIA, 75005 Paris, France
f AgroParisTech, UMR 518 MIA, 75005 Paris, France
g INRA, UMR 1282 Infectiologie et Santé Publique, 37380 Nouzilly, France

ABSTRACT Infections with *Salmonella enterica* belong to the most prominent causes of food poisoning and infected fruits and vegetables represent important vectors for salmonellosis. Recent evidence indicates that plants recognize *S. enterica* and raise defense responses. Nonetheless, the molecular mechanisms controlling the interaction of *S. enterica* with plants are still largely unclear. Here, we show that flagellin from *S. enterica* represents a prominent pathogen-associated molecular pattern (PAMP) in *Arabidopsis thaliana*, which induces PAMP-triggered immunity (PTI) via the recognition of the flg22 domain by the receptor kinase FLS2. The *Arabidopsis fls2* mutant shows reduced though not abolished PTI activation, indicating that plants rely also on recognition of other *S. enterica* PAMPs. Interestingly, the *S. enterica* type III secretion system (T3SS) mutant *prgH*– induced stronger defense gene expression than wild-type bacteria in *Arabidopsis*, suggesting that T3SS effectors are involved in defense suppression. Furthermore, we observe that *S. enterica* strains show variation in the flg22 epitope, which results in proteins with reduced PTI-inducing activity. Altogether, these results show that *S. enterica* activates PTI in *Arabidopsis* and suggest that, in order to accomplish plant colonization, *S. enterica* evolved strategies to avoid or suppress PTI.

Key words: *Salmonella enterica*; *Arabidopsis thaliana*; flagellin; FLS2; immunity.

INTRODUCTION

Salmonella is one of the most widespread food-borne pathogens and salmonellosis represents an important health concern worldwide. Several reports indicate that plants can host human pathogens and *Salmonella enterica* was shown to colonize a number of plant species, such as *Arabidopsis*, *Medicago sativa* (alfalfa), *Solanum lycopersicum* (tomato), and various leafy green vegetables (Cooley et al., 2003; Iniguez et al., 2005; Schikora et al., 2008; Berger et al., 2009; Kroupitski et al., 2009; Barak et al., 2011; Berger et al., 2011; Golberg et al., 2011; Schikora et al., 2011; Barak and Schroeder 2012). Nevertheless, *S. enterica* subspecies and serovars display differences in their interaction with plants and the plant immune system was shown to control endophytic colonization (Iniguez et al., 2005; Schikora et al., 2008; Berger et al., 2009; Berger et al., 2011; Barak and Schroeder 2012).

Plants rely on a sophisticated immune system to appropriately respond to pathogenic or beneficial microbes. An early layer in plant immunity relies on the detection of pathogen (or microbe)-associated molecular patterns (PAMPs) by membrane-resident receptor kinases and is called PAMP-triggered immunity (PTI) (Zipfel, 2009). The best-studied PAMP perception system is that of FLS2 (flagellin sensing 2)-mediated recognition of the flg22 epitope at the N-terminal region of bacterial flagellin (Gomez-Gomez and Boller, 2000; Zipfel

[1] To whom correspondence should be addressed. E-mail ana.garcia@evry.inra.fr, tel. +33 (0)1 60 87 45 22, fax +33 (0)1 60 87 45 10.

doi:10.1093/mp/sst145, Advance Access publication 5 November 2013

Received 21 August 2013; accepted 9 October 2013

et al., 2004). Although PAMPs are considered to be widely conserved, several reports have shown that certain bacteria evolved divergent flagellins leading to reduced PTI activation (Felix et al., 1999; Sun et al., 2006; Cai et al., 2011; Clarke et al., 2013). PAMP recognition leads to a series of early and late responses, including activation of kinase cascades, production of reactive oxygen species (ROS), induction of defense genes, and accumulation of defense hormones (Gomez-Gomez and Boller, 2000; Asai et al., 2002; Zipfel et al., 2004; Boudsocq et al., 2010). The activation of PTI is central to host immunity as it stops colonization by most potential pathogens (Zipfel, 2009). Nevertheless, successful pathogens use secretion systems to deliver effectors to the host cell that interfere with PTI and allow disease progression. Plants combat these adapted pathogens with a versatile family of intracellular NB-LRR (nucleotide binding-leucine rich repeat) receptors that recognize effectors and initiate so-called effector-triggered immunity (ETI) (Heidrich et al., 2012). NB-LRR perception of pathogen effectors amplifies PTI responses and typically leads to the accumulation of the defense hormone salicylic acid (SA) and localized cell death (Heidrich et al., 2012). Whereas this is true for biotrophic pathogens, necrotrophic pathogens that feed on dead tissue induce defense responses mediated by the hormones jasmonic acid (JA) and ethylene (ET) and many other hormones have been found to influence plant–pathogen interactions (Robert-Seilaniantz et al., 2011).

An increasing number of reports are expanding our knowledge about the interaction of plants with *S. enterica* and other human pathogens (Barak and Schroeder, 2012; Schikora et al., 2012; Goudeau et al., 2013). *S. enterica* was shown to attach to and invade plant tissues through natural openings and wounds (Schikora et al., 2008; Berger et al., 2009; Kroupitski et al., 2009). Furthermore, *S. enterica* serovar Typhimurium (*S.* Typhimurium) can multiply to different levels and persist in plant tissues (Cooley et al., 2003; Iniguez et al., 2005; Schikora et al., 2008; Berger et al., 2011). *S. enterica* codes for a T3SS-1 (type III secretion system-1) expressed at the extracellular stage and a T3SS-2 that is induced after internalization into animal cells (Galan, 2009). The T3SS-1 and T3SS-2 are encoded by the *Salmonella* pathogenicity islands 1 (SPI-1) and SPI-2, respectively, and facilitate the secretion of a suite of effectors important for animal pathogenicity (Galan, 2009). *S.* Typhimurium mutants in either secretion system trigger enhanced immune responses and show reduced proliferation in plants, suggesting that T3SS effectors participate in the plant colonization process (Schikora et al., 2011; Shirron and Yaron, 2011). On the plant side, *S. enterica* inoculation leads to the activation of various defense responses, such as stomatal closure, ROS production, activation of mitogen-activated protein kinases (MAPKs), and defense gene expression (Schikora et al., 2008, 2011; Shirron and Yaron, 2011; Roy et al., 2013). Furthermore, pretreatment with the ET precursor ACC reduces *S.* Typhimurium colonization of *M. sativa* roots (Iniguez et al., 2005) and *Arabidopsis* seedlings impaired in ET or JA signaling displayed increased susceptibility to *S.* Typhimurium (Schikora et al., 2008). These observations indicate that plants recognize and activate defense responses against *S. enterica*, although the molecular mechanisms are not known.

In animals, the recognition of *S. enterica* through its O antigen, reflecting variation in the lipopolysaccharide (LPS), and its H antigen, reflecting variation in flagellin, is essential for innate immunity activation (Broz et al., 2012). Most *Salmonella* carry two flagellin-encoding genes, *fliC* and *fljB*, and have the capability of 'phase variation' through which they alternate between the expression of the two flagellar genes (Silverman and Simon, 1980). In plants, a recent study suggested that the *S. enterica* O antigen could constitute a new PAMP recognized by *Arabidopsis* (Berger et al., 2011). Furthermore, an *S.* Typhimurium mutant that lacks the *fliC* and *fljB* flagellin-encoding genes showed increased root colonization in alfalfa, wheat, and *Arabidopsis* (Iniguez et al., 2005), suggesting that *S.* Typhimurium flagellins are recognized in plants and elicit a defense response that reduces endophyte colonization. In this study, we demonstrate that *S. enterica* activates PTI in *Arabidopsis*. We show that the *Arabidopsis* flagellin receptor FLS2 recognizes the *S. enterica* flg22 peptide, conserved in *fliC* and *fljB*-encoded flagellins. The absence of a functional FLS2 reduces but does not completely abolish PTI activation in response to *S. enterica*, demonstrating that FLS2 represents an important but not unique *S. enterica* PAMP receptor.

RESULTS

Transcriptome Analysis

We previously showed that *S.* Typhimurium triggers a significant transcriptional reprogramming in *Arabidopsis* (Schikora et al., 2011). By comparing the transcriptional changes in response to *S.* Typhimurium wild-type and the *prgH* mutant at 24 h post inoculation, we observed that *prgH*$^{-}$ triggered a stronger induction of defense genes than wild-type. *prgH* encodes a component of the T3SS-1 needle complex and its mutation causes defects in animal cell invasion (Behlau and Miller, 1993; Pawelek et al., 2002). SPI-1 genes are known to be induced by high osmolarity, low oxygen levels, and short-chain fatty acids (Hautefort et al., 2003) and we therefore wondered whether *prgH* is expressed when in contact with plants. To this end, we used *S.* Typhimurium carrying the promoter–reporter fusions P*prgH-gfp*+ and the constitutively expressed P*rpsM-gfp*+ to inoculate liquid MS alone or containing *Arabidopsis* seedlings as performed for the microarray analysis and monitored GFP signals by fluorescence microscopy. In the medium without seedlings, we observed strong P*rpsM-gfp*+ and weak P*prgH-gfp*+ derived signals, while *S.* Typhimurium wild-type bacteria showed no GFP signal as expected (Figure 1A). Interestingly, incubation of P*prgH-gfp*+ and P*rpsM-gfp*+ in medium containing *Arabidopsis* seedlings showed strongly fluorescing bacteria on the surface of *Arabidopsis* roots (Figure 1B and Supplemental Figure 1A).

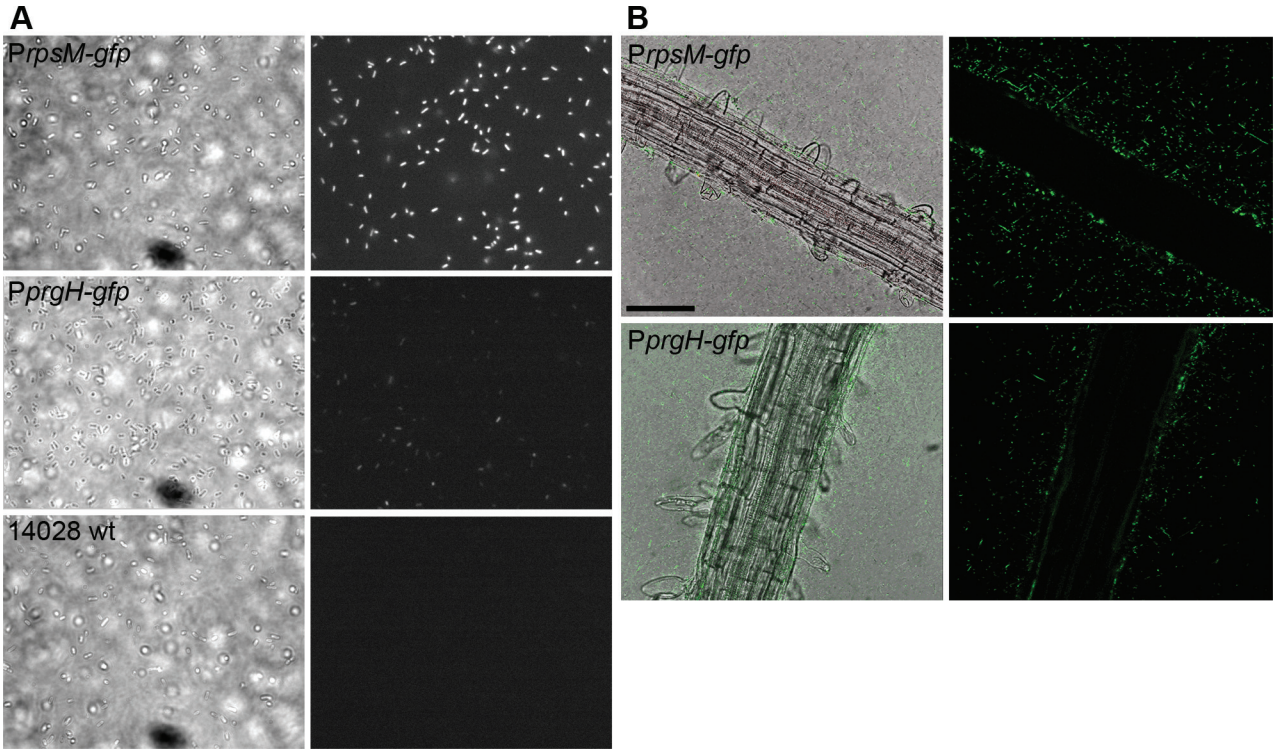

Figure 1. The *prgH* gene is expressed in *S.* Typhimurium cells in contact with *Arabidopsis* roots.
Liquid MS media alone **(A)** or containing 2-week-old *Arabidopsis* seedlings **(B)** were inoculated with *S.* Typhimurium carrying chromosomal insertions of the promoter fusions P*prgH-gfp*+ and P*rpsM-gfp*+, or with the *S.* Typhimurium wild-type strain 14028s. GFP fluorescence was monitored in an epifluorescence (A) and a confocal microscope (B) 1 h after inoculation. Bar is 75 μm.

These results indicate that the *prgH* gene is expressed under these cultivation conditions and suggest that *prgH* expression is induced by *Arabidopsis* factors that are either secreted or surface exposed.

To further characterize the interaction between *Arabidopsis* and *S.* Typhimurium, we decided to perform a more exhaustive transcriptome analysis. Two-week-old seedlings were challenged with *S.* Typhimurium wild-type or the *prgH* mutant, samples were collected at 2, 4, 6, 12, and 24 h post inoculation with bacterial or mock (10 mM $MgCl_2$) solutions and the resulting probes of two independent biological replicates were hybridized to CATMA (Complete Arabidopsis Transcriptome Micro Array) arrays version 5. This analysis revealed 3849 genes that were differentially expressed in at least one time point and one bacterial treatment. For each time point, we decided to focus on the genes showing at least a two-fold change (log ratio >1 or <–1) for further analysis, which produced a list of 1427 genes. We were intrigued by the dynamic behavior of the transcriptional response induced by *Salmonella* (Figure 2). The analysis of the variance and correlation coefficients between biological replicates allowed us to conclude that this observation is not due to any experimental error and therefore likely reflects the dynamics of the interaction between *Arabidopsis* and *S.* Typhimurium.

Looking at the induced genes, a first striking observation was that both bacteria triggered the greatest changes in the transcriptome at 2 h post inoculation (Figure 2A). At this time point, *prgH* induced a group of 455 genes, which contains almost entirely the genes induced by the wild-type strain (262 of the 275 wild-type-induced genes) (Figure 2A). By looking at the overlap between the genes induced at 2 h with those induced at later time points, we observed that many of the early-induced genes are up-regulated throughout the entire experiment (Supplemental Figure 2A and 2B). Of the 275 genes induced by wild-type at 2 h, we found 29% being up-regulated at 24 h, whereas, among the 455 *prgH*-induced genes at 2 h, a higher percentage stayed up-regulated at the different time points and 35% of the initial genes were still induced at 24 h. Given that most of the genes induced at 4 h were included in the gene set induced at 2 h, we decided to assess the overlap between the 2-h time point with the later time points of the kinetic to compare early and late responses. This analysis revealed that 26% of the *prgH* early-induced genes stayed up-regulated throughout the 24-h period analyzed, whereas this number dropped to 12% for the wild-type-induced genes (Supplemental Table 1). In the next step, we assessed the gene ontology (GO) enrichment among the up- and down-regulated genes using the Bio-Array Resource for Plant Biology (http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi) and the GO classification source (Provart et al., 2003). The analysis of the up-regulated genes showed that the GO terms 'stress response' and

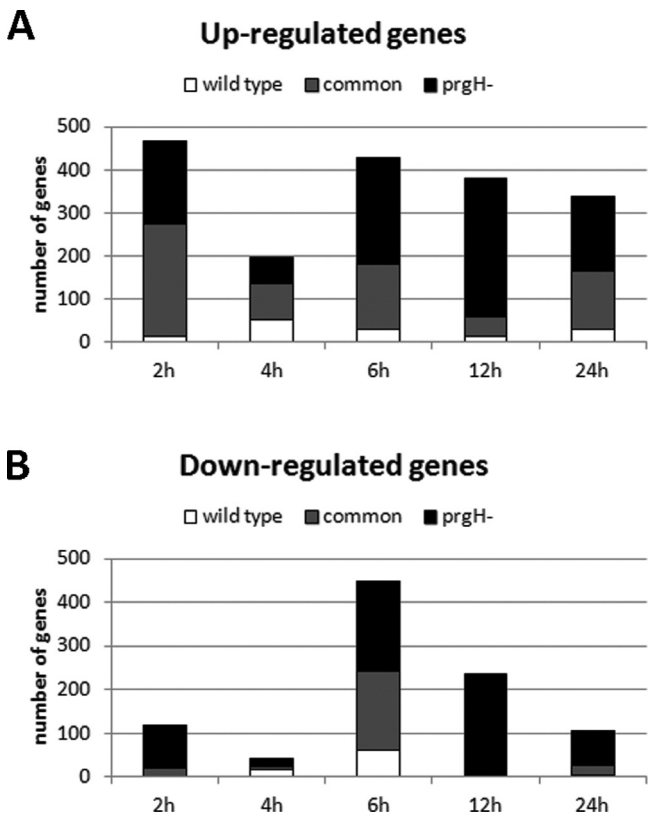

Figure 2. Number of differentially expressed genes in *Arabidopsis* seedlings in response to *S.* Typhimurium.

Number of up-regulated **(A)** and down-regulated **(B)** genes by *S.* Typhimurium wild-type and by the *prgH*⁻ mutant. Represented in white are genes regulated only by wild-type treatment, in black are genes regulated only by *prgH*⁻, and in gray are genes that are regulated by both bacterial treatments.

'response to abiotic or biotic stimulus' were overrepresented at all time points and treatments (Supplemental Table 2A). In summary, these results led us to conclude that at all time points of the analysis *Arabidopsis* reacts to *S.* Typhimurium *prgH* with the reprogramming of a greater number of stress-related genes than it does in response to *S.* Typhimurium wild-type. The differences in the responses could be due to reduced gene induction or to gene repression by the wild-type strain. The *S.* Typhimurium *prgH* mutant was shown to proliferate at lower levels than wild-type bacteria in 5-week-old soil-grown plants (Schikora et al., 2011) and, in this study, we detected no significant differences between wild-type and *prgH* proliferation levels in *Arabidopsis in vitro*-grown seedlings (Supplemental Figure 1B), which suggests that the enhanced defense gene induction observed at 24 h after *prgH* inoculation is not related to differences in bacterial titers.

Among the repressed genes, we observed that both bacterial treatments provoke a peak of gene repression at 6 h post inoculation, with 242 genes being repressed by the wild-type bacteria and 385 genes by *prgH* (Figure 1B). An important percentage of the down-regulated genes at 6 h (180 genes) are repressed by both *S.* Typhimurium treatments (Figures 1B, and Supplemental Figure 2C and 2D), suggesting that the observed gene repression is a general plant response to *Salmonella* perception. Given that the biggest number of repressed genes occurred at 6 h after challenge with both bacterial treatments and that these gene lists presented an important overlap with the genes repressed at the other time points (Supplemental Figure 2C and 2D), we decided to focus on the 6-h time point for the GO term enrichment analysis. Using the GO classification, we found that 'electron transport or energy pathways' is the biological process most overrepresented in both gene lists (Supplemental Table 2B). We further analyzed the list of repressed genes for the GO enrichment using the MapMan annotation and this analysis revealed 'photosynthesis' as the most affected biological process (Supplemental Table 2C).

To validate the transcriptional changes observed in the microarray approach, we selected genes for qPCR analysis using three new independent biological replicates. Genes were selected according to their expression profile (induction by *S.* Typhimurium wild-type and *prgH* at early time points or differences between wild-type and *prgH* induced expression at late time points) or for being marker genes for defense signaling pathways (Tables 1, 2, and Supplemental Tables 3 and 4). From the 26 induced genes tested, all showed a qPCR profile that correlated with the microarray results (Supplemental Figure 3). Importantly, we could confirm for several genes that *S.* Typhimurium wild-type bacteria induces higher expression at 2 h than 4 h post inoculation (*SID2*, *WRKY33*, *NUDT7*, *NHL3*, *CBP60g*, *AT5G35735*) and for all except one gene (*PUB23*) we could confirm a higher expression at 24 h post treatment with *prgH* than with wild-type. Altogether, through the analysis of five independent biological replicates (two used for the microarray and three used for the qPCR), we could conclude that *S.* Typhimurium triggers a dynamic transcriptional response in *Arabidopsis*. This response includes the early induction of defense genes observed at 2 h and to a lesser extent at 4 h and then a late defense response which is stronger after treatment with *prgH* than wild-type.

Salmonella Typhimurium Induces Typical PAMP-Induced Transcriptional Reprogramming in *Arabidopsis*

We compared our gene expression data with analyses done in *Arabidopsis* in response to other bacterial or PAMP treatments. One of the first genome-wide analyses of PAMP-induced transcriptional reprogramming used ATH1 chips to assess the responses occurring at 30 min after flg22 treatment in L*er*-0 seedlings (Zipfel et al., 2004). This study identified 966 up-regulated genes and 202 down-regulated genes, which were called FLAgellin-REsponsive (FLARE) genes. Thilmony and co-workers used 4-week-old leaves to study the responses to bacterial treatments at 7, 10, and 24 h post bacterial infiltration and found 2800 differentially regulated genes (Thilmony et al., 2006). With the use of specific *Pseudomonas*

Table 1. Microarray Profile of the Genes Tested by qPCR.

		wild type					*prgH*-						
AGI	Description	2h	4h	6h	12h	24h	2h	4h	6h	12h	24h	PIG	FLARE
AT1G33960	AIG1	0.48	0.30	0.52	-0.06	0.12	0.07	0.11	**1.05**	**1.67**	**1.41**		
AT1G66090	Disease resistance protein (TIR-NBS class), putative	**1.24**	0.46	0.51	0.32	0.08	**1.84**	**1.14**	**1.50**	**1.72**	**1.39**		
AT1G74710	ICS1/SID2	**1.84**	**0.82**	0.45	0.14	0.08	**2.13**	**1.18**	**1.52**	**1.68**	**1.16**		
AT2G35980	YLS9	**1.67**	0.54	0.49	0.24	-0.04	**2.09**	**1.65**	**1.32**	**1.81**	**1.67**	+	+
AT2G38470	WRKY33	**1.52**	0.42	**0.79**	0.48	0.25	**2.04**	**1.10**	**1.47**	**1.40**	**1.36**		+
AT3G22600	Protease inhibitor/seed storage/lipid transfer protein	–0.05	0.21	–0.47	0.09	**-0.83**	–0.27	**1.46**	**0.85**	**1.95**	**1.34**		
AT3G25882	NIMIN-2	0.44	0.65	0.18	0.33	0.31	0.44	**0.67**	**1.51**	**1.50**	**1.43**		
AT3G52430	PAD4	**1.21**	0.58	0.34	–0.06	0.17	0.42	**0.90**	**1.25**	**1.44**	**1.45**		
AT3G61190	BAP1	0.47	0.62	0.49	0.19	0.58	**0.78**	**0.90**	**1.48**	**1.47**	**1.74**		
AT4G12720	AtNUDT7	**1.56**	0.30	0.39	0.24	0.23	**1.28**	**0.93**	**1.42**	**1.66**	**1.33**		+
AT5G06320	NHL3	**1.61**	0.34	**1.08**	**0.96**	**1.01**	**1.80**	**1.25**	**1.84**	**2.29**	**2.05**		+
AT2G46400	WRKY46	0.64	0.05	0.18	0.03	–0.02	0.53	0.27	**1.20**	**1.14**	**0.86**		
AT5G13320	PBS3	**0.89**	0.28	0.45	0.22	0.29	**0.72**	**0.79**	**1.40**	**1.63**	**1.27**		
AT1G14540	Anionic peroxidase, putative	**1.86**	**1.14**	**1.28**	**0.73**	0.37	**2.38**	**1.83**	**1.60**	**1.89**	**1.20**	+	+
AT3G48090	EDS1	**0.71**	0.36	0.53	0.15	0.12	**0.77**	**0.61**	**0.86**	**1.14**	**0.76**		
AT1G18570	MYB51	**1.31**	0.42	0.26	0.06	0.21	**1.59**	**0.72**	**1.01**	**1.19**	**0.67**	+	+
AT5G26920	CBP60g	**2.00**	**0.95**	0.51	0.36	**0.90**	**2.18**	**1.70**	**1.59**	**1.77**	**1.68**	+	+
AT2G23810	TET8 (TETRASPANIN8)	0.63	0.22	**0.79**	0.55	**0.68**	**0.70**	0.56	**1.89**	**1.58**	**1.53**		
AT3G16530	Legume lectin family protein	**1.76**	**1.46**	**0.62**	**0.63**	0.22	**2.20**	**1.52**	**1.14**	**1.51**	**0.82**	+	+
AT5G35735	Auxin-responsive family protein	**1.37**	0.31	–0.15	0.17	0.24	**1.54**	**1.11**	–0.17	**1.32**	**1.03**		+
AT3G23250	MYB15	**2.68**	**1.87**	**1.78**	**0.96**	**1.38**	**2.94**	**2.31**	**2.19**	**2.25**	**2.17**	+	+
AT2G35930	PUB23	**1.85**	**1.57**	**1.18**	**0.91**	0.61	**2.30**	**1.65**	**1.71**	**1.10**	**1.02**	+	+
AT1G10340	Ankyrin repeat family protein	**1.29**	**0.83**	**1.36**	0.43	**1.41**	**0.76**	**0.72**	**1.47**	**1.38**	**1.92**		
AT1G02360	Chitinase, putative	**1.45**	**1.20**	**0.88**	0.47	0.14	**1.63**	**0.88**	**1.46**	**1.67**	**1.07**	+	+
AT3G56400	WRKY70	0.33	0.23	0.27	0.11	0.00	0.32	0.44	**1.08**	**1.63**	**1.01**		
AT3G04720	PR4	**0.70**	**1.32**	**0.96**	0.53	**0.75**	**0.87**	**1.32**	**1.02**	**1.24**	**1.64**		

Values represent expression changes with respect to the mock treatment and are given as $\log_2$. The genes that are part of the PIG and FLARE lists are marked on the right. Expression changes in bold correspond to genes differentially expressed at the significance threshold of Bonferroni $P < 0.05$.

syringae and *Escherichia coli* mutants, the authors defined a set of PAMP-induced genes (PIGs). Given the high overlap between the *prgH* and wild-type-induced genes at 2 h, we chose the '*prgH*/2h' gene set for a comparative analysis. We observed that, despite the differences in ecotype, age of plants, and growth conditions, there was an important overlap between the different gene lists of induced genes. From the 455 genes regulated by *prgH*, 109 genes (24%) were part of the PIGs, 190 genes (41.8%) were included in the FLAREs, and 69 of these genes were included in all three gene lists (Supplemental Table 3).

Among the overlapping genes, we found known PTI marker genes and we chose several for validation by qPCR (Table 1 and Supplemental Table 3). Among the selected genes were included: *CBP60g* encoding a calmodulin-binding protein that contributes to PAMP-induced SA accumulation, *PUB23* encoding a U-box type E3 ubiquitin ligase that negatively regulates PTI, and genes coding for the transcription factors MYB51, necessary for the induction of callose deposition and glucosinolate metabolism, and WRKY33, a MPK3/MPK6 target that regulates the expression of pathogen-responsive genes (Trujillo et al., 2008; Clay et al., 2009; Wang et al., 2009; Birkenbihl et al., 2012; Li et al., 2012; O'Brien et al., 2012; Logemann et al., 2013). Using three independent biological replicates, we confirmed by qPCR that some of these genes are strongly induced at 2 h after wild-type and *prgH* treatments (Table 1 and Supplemental Figure 3). Furthermore, as observed in the transcriptome analysis, all genes showed stronger expression in response to *prgH* at later time points. Therefore, these results suggest that *S.* Typhimurium triggers the reprogramming of PTI-regulated genes at early time points, which is reduced by *S.* Typhimurium wild-type at later time points but not by *S.* Typhimurium *prgH*.

Table 2. Microarray Expression of Marker Genes of the Salicylic Acid (SA), Jasmonic Acid (JA), Ethylene (ET), ABA, and Auxin Hormone Pathways in *Arabidopsis*.

		Wild type					*prgH-*					
AGI	Description	2h	4h	6h	12h	24h	2h	4h	6h	12h	24h	Pathway
AT1G74710	ICS1/SID2	**1.84**	**0.82**	0.45	0.14	0.08	**2.13**	**1.18**	**1.52**	**1.68**	**1.16**	SA
AT3G48090	EDS1	**0.71**	0.36	0.53	0.15	0.12	**0.77**	**0.61**	**0.86**	**1.14**	**0.76**	SA
AT3G52430	PAD4	**1.21**	0.58	0.34	–0.06	0.17	0.42	**0.90**	**1.25**	**1.44**	**1.45**	SA
AT4G39030	EDS5	–0.02	0.14	–0.08	0.06	0.07	0.13	0.09	–0.19	**0.77**	0.06	SA
AT5G13320	PBS3	**0.89**	0.28	0.45	0.22	0.29	**0.72**	**0.79**	**1.40**	**1.63**	**1.27**	SA
AT1G02450	NIMIN-1	0.09	0.37	0.16	–0.05	0.23	0.18	0.27	**1.03**	0.55	0.32	SA
AT3G25882	NIMIN-2	0.44	0.65	0.18	0.33	0.31	0.44	**0.67**	**1.51**	**1.50**	**1.43**	SA
AT5G26920	CBP60g	**2.00**	**0.95**	0.51	0.36	**0.90**	**2.18**	**1.70**	**1.59**	**1.77**	**1.68**	SA
AT3G56400	WRKY70	0.33	0.23	0.27	0.11	0.00	0.32	0.44	**1.08**	**1.63**	**1.01**	SA
AT2G14610	PR1	0.11	0.06	**0.92**	0.10	0.19	–0.01	–0.16	0.09	0.36	**1.33**	SA
AT3G45140	LOX2	0.56	**1.35**	**1.08**	**0.71**	**1.12**	0.33	**0.67**	0.62	–0.19	0.57	JA
AT1G17420	LOX3	0.62	0.00	0.39	–0.13	0.14	**1.18**	0.41	0.68	**1.07**	0.53	JA
AT1G72520	LOX4	**0.94**	–0.04	0.13	0.18	0.08	**1.69**	**0.71**	**0.97**	**1.52**	0.60	JA
AT5G42650	AOS	0.38	–0.09	–0.47	0.05	–0.46	**0.79**	–0.03	–0.30	–0.09	**–1.06**	JA
AT3G25760	AOC1	**1.00**	0.27	–0.52	0.06	0.39	**1.52**	0.18	–0.29	**–0.75**	–0.48	JA
AT3G25780	AOC3	**1.26**	0.18	0.27	0.08	–0.22	**1.78**	0.50	**0.75**	**0.98**	–0.04	JA
AT1G76680	OPR1	**1.23**	–0.41	–0.36	0.33	**–0.78**	**1.55**	0.25	0.48	**0.84**	–0.52	JA
AT2G06050	OPR3	**0.74**	0.07	**–0.59**	0.14	0.07	**0.90**	0.12	**–0.79**	–0.09	–0.09	JA
AT5G44420	PDF1.2	–0.06	0.25	–0.18	0.15	0.57	0.07	0.13	–0.03	**0.70**	0.37	JA
AT5G13220	JAZ10	0.61	–0.11	0.21	0.28	0.22	**0.63**	0.19	0.43	**0.68**	0.14	JA
AT4G23600	CORI3	0.39	0.27	–0.04	0.05	0.16	**0.64**	0.08	0.16	0.00	0.01	JA
AT1G19640	JMT	–0.14	0.09	**–0.81**	–0.44	0.06	–0.07	–0.06	–0.13	–0.20	–0.14	JA
AT5G47220	ERF2	**0.72**	0.45	0.23	0.50	0.30	**1.11**	**0.78**	0.62	0.31	**0.90**	ET
AT4G17500	ERF1	0.57	0.29	–0.16	0.38	0.30	**0.92**	0.45	0.10	**0.72**	0.65	ET
AT5G47230	ERF5	**0.73**	0.33	0.42	0.02	0.02	**1.14**	0.57	**0.84**	**0.63**	0.50	ET
AT1G28370	ERF11	**0.74**	0.09	0.24	0.31	0.24	**1.23**	0.24	**0.73**	0.31	**0.71**	ET
AT3G50260	ERF011/CEJ1 (COOPERATIVELY REGULATED BY ETHYLENE AND JASMONATE 1)	**0.96**	0.55	**0.91**	0.05	**0.85**	**0.86**	**0.79**	**1.44**	**1.27**	**1.69**	ET
AT2G27050	EIL1	0.36	0.00	**–0.93**	0.25	0.27	0.18	–0.06	**–1.19**	**–0.71**	–0.63	ET
AT5G35220	EGY1 (ETHYLENE-DEPENDENT GRAVITROPISM-DEFICIENT AND YELLOW-GREEN 1)	–0.51	–0.55	**–1.34**	–0.07	–0.51	–0.42	–0.36	**–1.74**	**–1.23**	**–1.00**	ET
AT1G05010	EFE (ethylene forming enzyme)	**1.68**	**0.96**	**–0.86**	**0.74**	**0.82**	**2.06**	**0.88**	–0.44	–0.61	**0.76**	ET
AT2G41430	ERD15	**1.16**	0.31	**0.64**	**0.66**	**0.76**	**1.05**	0.16	**0.78**	**0.77**	0.58	ABA
AT5G51070	ERD1	0.19	–0.05	0.10	0.18	**0.70**	0.20	–0.07	0.50	0.49	**0.81**	ABA
AT5G15970	KIN2	0.22	0.22	0.05	**–1.05**	–0.44	–0.02	0.40	0.59	–0.35	**–0.70**	ABA
AT3G30775	ERD5	0.50	0.31	**–0.83**	0.14	0.61	0.55	–0.35	**–1.93**	**–1.45**	–0.06	ABA
AT3G26744	ICE1	–0.29	–0.19	**–0.69**	0.04	–0.16	–0.15	–0.04	**–1.21**	**–0.72**	**–0.72**	ABA
AT2G38120	AUX1	–0.04	–0.10	**–0.70**	0.11	–0.26	–0.17	0.01	**–0.78**	**–0.89**	–0.34	Auxin
AT2G01420	PIN4	–0.14	–0.11	**–1.18**	0.09	–0.23	–0.31	–0.05	**–1.34**	**–1.17**	–0.38	Auxin
AT1G12820	AFB3	–0.05	–0.49	**–0.85**	0.27	–0.05	–0.08	0.34	**–0.85**	–0.08	**–1.22**	Auxin
AT5G62000	ARF2	0.11	–0.09	–0.43	0.09	–0.09	0.19	–0.02	**–0.77**	–0.34	–0.05	Auxin
AT3G23030	IAA2	0.48	0.56	**0.84**	**0.89**	**1.09**	0.33	0.32	**0.99**	**0.72**	**1.26**	Auxin
AT1G04550	IAA12	–0.55	–0.17	–0.14	0.06	–0.17	**–0.63**	–0.15	–0.33	–0.10	–0.24	Auxin
AT2G33310	IAA13	0.16	–0.11	**–1.06**	0.14	–0.02	–0.13	0.26	**–1.09**	**–0.69**	–0.36	Auxin
AT3G04730	IAA16	0.27	–0.07	**–0.74**	0.10	0.10	0.04	–0.20	**–0.82**	**–0.81**	–0.12	Auxin

Table 2. Continued

AGI	Description	Wild type					*prgH-*					Pathway
		2h	4h	6h	12h	24h	2h	4h	6h	12h	24h	
AT3G15540	IAA19	**0.68**	0.07	–0.05	0.15	0.20	0.55	0.50	0.09	0.26	0.31	Auxin
AT2G04160	AIR3 (Auxin-induced in root cultures 3); subtilase	0.17	0.39	0.42	**0.71**	0.30	0.43	0.48	0.60	**0.63**	**0.76**	Auxin
AT3G07390	AIR12 (Auxin-induced in root cultures 12)	**2.38**	**0.93**	0.11	**1.15**	**1.15**	**2.63**	**1.56**	**1.02**	**1.57**	**2.00**	Auxin
AT4G37390	YDK1 (AUXIN UPREGULATED1, YADOKARI 1)	**1.16**	**1.07**	**1.15**	**1.61**	**1.06**	**1.31**	0.57	**1.74**	**1.25**	**1.67**	Auxin
AT5G35735	Auxin-responsive family protein	**1.37**	0.31	–0.15	0.17	0.24	**1.54**	**1.11**	–0.17	**1.32**	**1.03**	Auxin
AT5G50760	Auxin-responsive family protein	–0.03	–0.02	0.31	0.16	0.31	–0.25	–0.11	**0.78**	**1.17**	**0.78**	Auxin
AT2G36210	Auxin-responsive family protein	**0.86**	**1.16**	0.49	0.48	0.61	**1.30**	0.34	**0.82**	0.15	0.63	Auxin
AT3G60690	Auxin-responsive family protein	0.59	0.06	–0.28	0.22	0.40	**0.94**	0.56	–0.05	0.24	0.10	Auxin
AT2G46690	Auxin-responsive family protein	–0.57	–0.03	0.14	–0.39	–0.26	**–0.91**	–0.43	0.08	–0.06	–0.26	Auxin
AT1G71090	Auxin efflux carrier family protein	0.12	0.02	–0.45	–0.07	0.07	–0.15	–0.17	**–0.78**	–0.47	–0.27	Auxin
AT4G12980	Auxin-responsive protein, putative	–0.27	–0.55	**–1.03**	–0.26	–0.22	–0.41	–0.35	**–0.77**	–0.50	–0.36	Auxin
AT4G38840	Auxin-responsive protein, putative	**–0.78**	–0.24	–0.10	–0.04	–0.17	**–1.17**	–0.57	–0.16	–0.43	–0.44	Auxin
AT4G34760	Auxin-responsive family protein	–0.63	–0.47	–0.44	–0.21	–0.52	**–0.84**	–0.18	–0.29	–0.61	–0.53	Auxin
AT1G20925	Auxin efflux carrier family protein	**–0.81**	–0.26	–0.03	–0.15	0.09	**–1.09**	**–0.79**	**–0.88**	–0.49	–0.09	Auxin

Values represent expression changes with respect to the mock treatment and are given as $\log_2$. Expression changes in bold correspond to genes differentially expressed at the significance threshold of Bonferroni $P < 0.05$.

The flg22 Peptide from *S. enterica* Is Recognized in *Arabidopsis* via FLS2 and Triggers PTI

Given the overlap between *S.* Typhimurium-induced transcriptional changes and the reprogramming induced by flg22 or PTI-inducing bacteria, we suspected that *Arabidopsis* recognizes PAMPs from *Salmonella*. Therefore, we decided to assess whether *Salmonella* flagellin perception could lead to PTI activation in *Arabidopsis.* We examined the *S.* Typhimurium flagellin sequences encoded by *fliC* and *fljB* and found that the N-terminal region shows 100% amino acid identity (Supplemental Figure 4). Furthermore, the flg22 domain from *S.* Typhimurium (hereafter called flg22-ST) is identical to that of multiple *S. enterica* serovars (Montevideo, Oranienburg, Enteritidis, Typhi, etc.) as well as other enterobacteria such as *E. coli*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Yersinia* spp., and *Shigella* spp. (Figure 3A). The flg22-ST sequence shows five amino acid changes with respect to the canonical flg22 sequence from *Pseudomonas aeruginosa* and the flg15 peptide corresponding to the flg22-ST sequence was previously found to possess 30% of the biological activity of the corresponding peptide from *P. aeruginosa* in tomato cells (Felix et al., 1999).

We synthesized the flg22-ST peptide and tested its activity in *Arabidopsis*. Despite the amino acid changes, flg22-ST induced various hallmarks of both early and late PTI to a similar extent to flg22 (Figure 3). In a seedling growth inhibition assay, flg22-ST showed similar activity as flg22 when applied at 1 μM and a minor reduction in peptide activity was observed at 100 nM (Figure 3B). Similarly, 100 nM flg22-ST triggered a slightly lower ROS production than flg22 (Figure 3C). To study the activation of the stress-induced MAPKs MPK3, MPK4, and MPK6, we used the anti-pTpY antibody that recognizes the activated forms of these three MAPKs. We observed similar activation of the three MAPKs 10 min after treatment with 1 μM flg22 or flg22-ST (Figure 3D). Furthermore, the induction of PTI marker genes assessed at 30 min and 2 h after treatment with 100 nM peptide showed similar induction (Figure 4). The accumulation of callose depositions is yet another PAMP-induced defense response revealed as aniline blue stained spots (Gomez-Gomez et al., 1999). We observed strong callose depositions in leaves 24 h after infiltration of 1 μM flg22-ST (Supplemental Figure 5). Finally, the PTI-inducing activity of the flg22-ST peptide was demonstrated by its capacity to induce immunity against *P. syringae* pv. *tomato* (*Pst*) strain DC3000 (Figure 3E). All PTI responses observed were FLS2-dependent and we therefore concluded that *Arabidopsis* recognizes the flg22-ST peptide in a similar way to flg22.

Salmonella Typhimurium Treatment Leads to the Activation of SA Biosynthesis and Signaling Genes

To further understand which pathways are involved in the defense responses against *S.* Typhimurium, we analyzed the transcriptome for markers genes of hormone pathways known to impact on defense signaling: JA, ET, SA, auxin, and ABA. Interestingly, when analyzing the 24-h time point, we observed that several of the genes that are strongly induced by *prgH* and that show lower or no induction by wild-type are SA marker genes (Table 2). We also observed the induction of

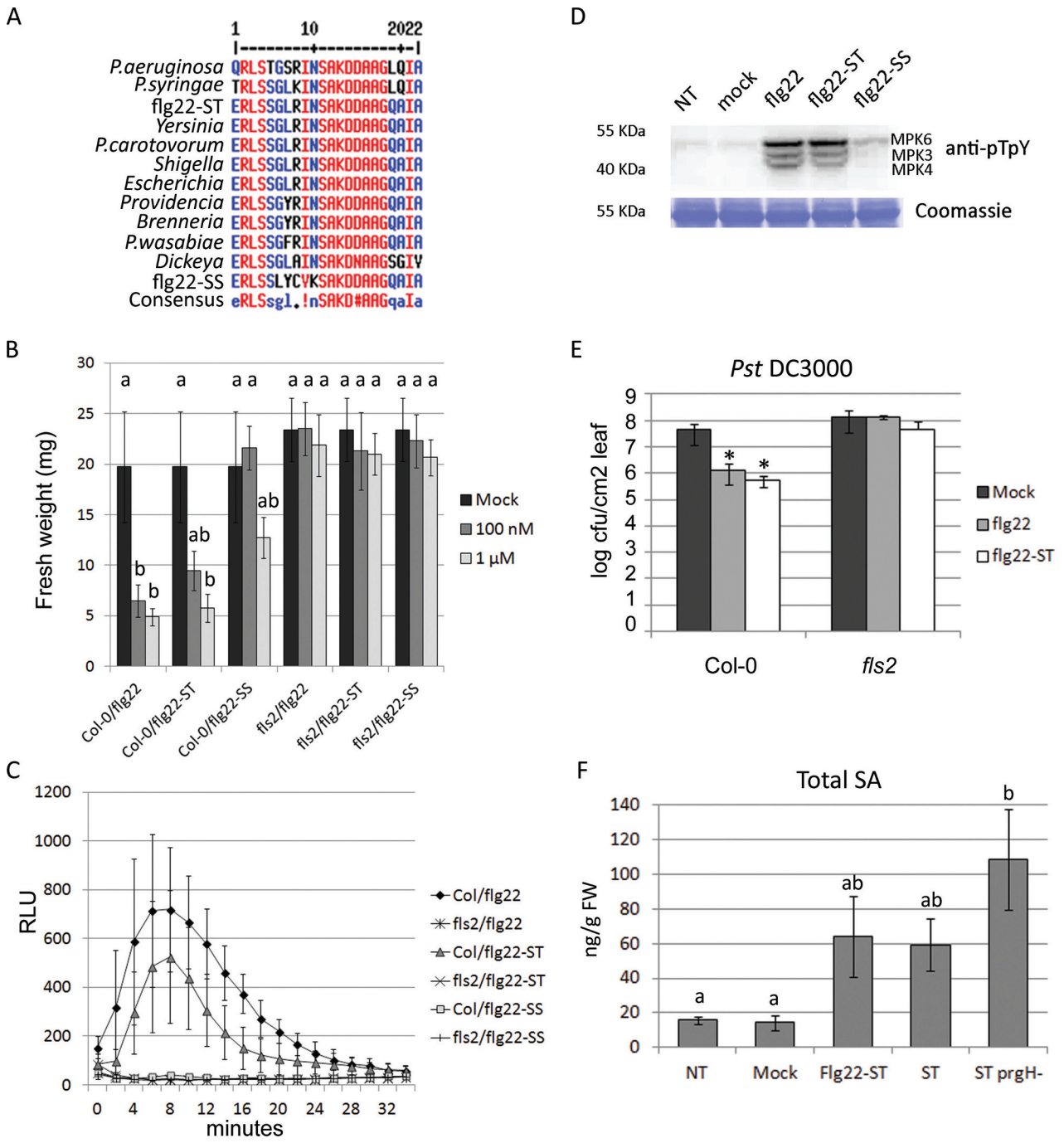

Figure 3. *S.* Typhimurium flg22 Activates PTI in *Arabidopsis*.

(A) Alignment of the flg22 amino acid sequences of *Pseudomonas aeruginosa*, *Pseudomonas syringae* pv. *syringae* DC3000, and various enterobacteria strains. The *S.* Typhimurium and *S.* Senftenberg peptides used in this study are flg22-ST and flg22-SS, respectively. The remaining sequences are named with the genus of each bacteria except for *Pseudomonas* and *Pectobacterium*, for which two sequences are presented and the species name is included. The sequences used are from: *P. aeruginosa* ATCC 700888, *P. syringae* pv. *tomato* str. DC3000, *S.* Senftenberg S05219 03, *S.* Typhimurium 14028S, *Yersinia rohdei* ATCC 43380, *Shigella dysenteriae* 1617, *Escherichia coli*, *Providencia sneebia* DSM 19967, *Brenneria* sp. EniD312, *Pectobacterium wasabiae* CFBP 3304, *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya dadantii* 3937.

(B) Seedling growth inhibition assay. Five-day-old Col-0 and *fls2* seedlings were transferred to liquid MS supplemented or not with the different flagellin peptides at 100 nM and 1 µM. Plants were weighted 7 d after treatment. Bars represent means ± SD (n = 12). Letters indicate significant differences based on a Kruskal & Wallis test, $\alpha \leq 0.05$.

(C) ROS production in Col-0 and *fls2* leaf discs in response to 100 nM flagellin peptides. Bars represent means ± SD (n = 12). RLU, relative light units. Differences between Col-0/flg22 and Col-0/flg22-ST are not significant based on the Kruskal & Wallis test, $\alpha \leq 0.05$.

(D) MAPK activation in Col-0 seedlings after treatment with 1 µM flagellin peptides or H_2O (mock) for 10 min. NT, non-treated.

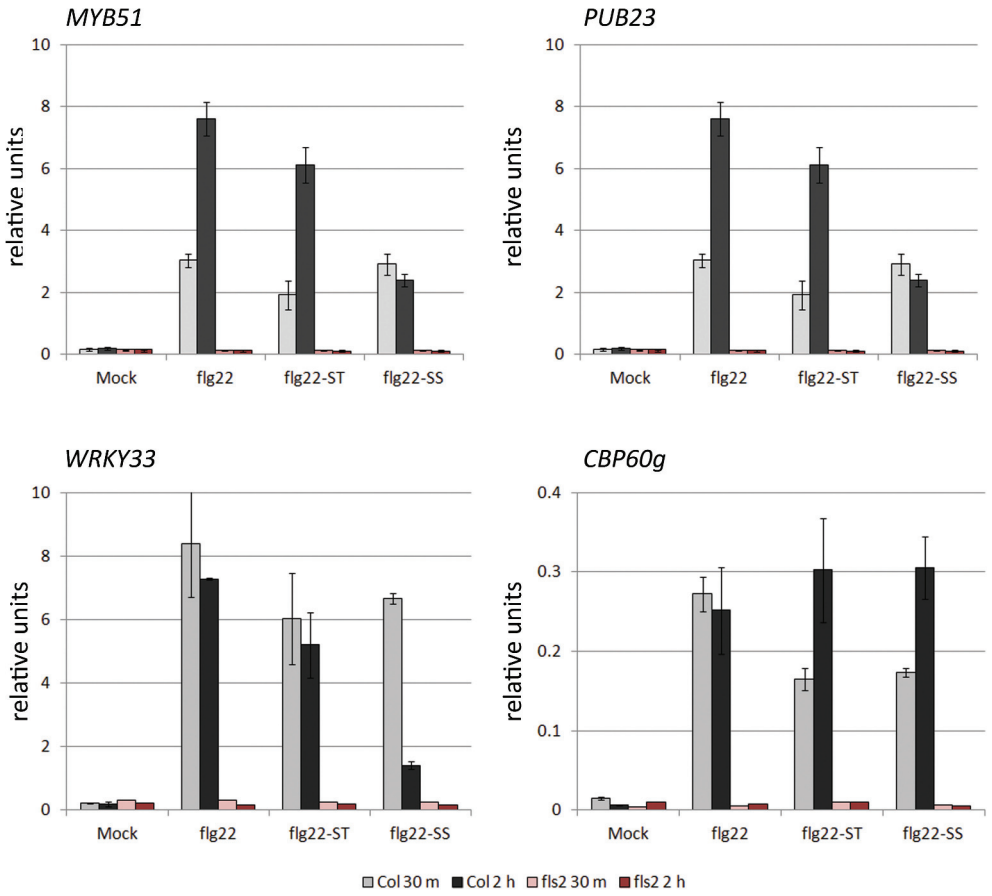

Figure 4. PTI Marker Gene Induction in Col-0 and *fls2* Seedlings at 30 min and 2 h in Response to 100 nM Flagellin Peptides.
Transcript accumulation is expressed relative to the average of the transcript level of two reference genes. Bars represent means of three replicates ± SD. Results are representative of three independent experiments.

JA and ET-responsive genes mainly at 2 h but the regulation of this group of genes was more variable. Interestingly, the ET and JA-responsive genes *PROPEP1*, *PROPEP2*, and *PROPEP3* encoding precursors of endogenous 'danger' signal peptides and their receptors *PEPR1* and *PEPR2* were not induced by *S.* Typhimurium (Huffaker and Ryan, 2007). These genes are part of a PTI amplification loop (Tintor et al., 2013) that seems to be absent in response to *S.* Typhimurium. Among the few ABA marker genes, *ERD15* is the one showing the highest induction in response to *S.* Typhimurium and codes for a negative regulator of ABA signaling (Aalto et al., 2012). Similarly, the regulation of auxin-responsive genes in response to *S.* Typhimurium is variable and the *YDK1* gene, showing the strongest induction, is a negative component of auxin signaling (Takase et al., 2004). The behavior of these marker genes suggested that the SA pathway is induced in response to *S.* Typhimurium. To confirm this observation, we chose several SA marker genes and validated their induction by *S.* Typhimurium using qPCR (Table 1 and Supplemental Figure 3). We selected genes coding for: SID2/ICS1, the main SA biosynthetic enzyme during ETI and PTI (Wildermuth et al., 2001; Tsuda et al., 2008); the lipase-like proteins EDS1 and PAD4 that interact *in vivo* and play crucial roles in SA-dependent resistance to biotrophic pathogens (Feys et al., 2001); NIMIN-2 that functions with NPR1, a central transducer of SA signaling in plant immunity (Weigel et al., 2005); WRKY70 and WRKY46 that have overlapping functions in SA-mediated resistance to *P. syringae* (Li et al., 2004; Hu et al., 2012); NUDT7 that negatively regulates EDS1-mediated immunity (Straus et al., 2010) and the acyl acid amido synthetase PBS3/GH3.12 that participates

(E) *S.* Typhimurium flagellin peptide flg22-ST triggers resistance against *Pst* DC3000. Leaves of 5-week-old Col-0 and *fls2* plants were pretreated with 1 µM flg22 peptides for 24 h and then infiltrated with a *Pst* DC3000 bacterial solution at 1×10^5 cfu ml^{-1}. Bacterial titers were determined 2 d post inoculation. Bars represent means ± SD ($n = 4$). Treatment with both flg22 peptides trigger a significant reduction in *Pst* DC3000 growth as compared to mock treatment (* p-value < 0.05).
(F) SA accumulation in *Arabidopsis* seedlings non-treated (NT) or treated for 24 h with H_2O (Mock), 1 µM flg22-ST, *S.* Typhimurium wild-type, or *prgH*$^-$ mutant. Bars are means of four replicates ± SD. Letters indicate significant differences based on a Kruskal & Wallis test, $\alpha \leq 0.05$.

in SA-mediated defenses against *P. syringae* (Jagadeeswaran et al., 2007; Nobuta et al., 2007). Based on these results, we decided to quantify SA accumulation in *Arabidopsis* seedlings 24 h after treatment with *S.* Typhimurium wild-type and *prgH*. In agreement with the transcriptional activation of SA biosynthetic and responsive genes, *S.* Typhimurium treatment led to a significant accumulation of SA, which was more pronounced after treatment with *prgH* (Figure 3F). SA accumulation is also observed during PTI (Tsuda et al., 2008) and we wondered whether flg22-ST could trigger SA accumulation in *Arabidopsis* seedlings. Treatment with flg22-ST 1 μM for 24 h induced SA accumulation to a similar extent as challenge of plants with *S.* Typhimurium wild-type (Figure 3F). This indicates that *S.* Typhimurium and flg22-ST trigger yet another PTI hallmark and suggests that the *S.* Typhimurium-induced SA accumulation could be due to flagellin recognition.

Salmonella-Induced PTI Is Partially Mediated by the *Arabidopsis* FLS2 Receptor

Knowing that *Arabidopsis* has the capability of recognizing *S.* Typhimurium flagellin, we wondered how much of the observed responses to *S.* Typhimurium are mediated by flagellin perception. One of the hallmarks of PTI is the induction of MAPK cascades and *S.* Typhimurium inoculation leads to the activation of MAPK cascades in mammals and plants (Schikora et al., 2008; Bruno et al., 2009). Using the anti-pTpY antibody, we compared MAPK activation at 10, 15, and 30 min after treatment with *S.* Typhimurium wild-type and *prgH* in Col-0 and *fls2* seedlings. Both bacterial treatments induced a similar activation of MPK3 and MPK6 in Col-0, but this activation was reduced in the absence of a functional FLS2 (Figure 5A). To assess whether the MAPK activation kinetics observed in response to *S.* Typhimurium was similar to that triggered by other phytophathogenic bacteria, we studied the activation of MAPKs in response to the well-characterized phytopathogenic bacteria *Pst* DC3000 and the T3SS mutant *Pst* DC3000 *hrcC*. Similarly to what we observed in response to *S.* Typhimurium, both *Pst* DC3000 and *Pst* DC3000 *hrcC* treatments activated MPK3 and MPK6 in *Arabidopsis* Col-0 wild-type plants and, to a lesser extent, in the *fls2* mutant (Figure 5B). Activated MPK4 was hardly detected in our Western blots and showed more variability among all treatments and biological replicates. In summary, similar MAPK activation kinetics and a significant reduction in *fls2* mutant plants were observed in response to both *S.* Typhimurium and *P. syringae*. To further assess whether the recognition of *S.* Typhimurium flagellin is important for defense induction, we tested the MAPK activation in response to an *S.* Typhimurium mutated in both flagellin-encoding genes *fliC* and *fljB*. This analysis revealed similar MAPK activation at 15 and 30 min after treatment with mutant and wild-type bacteria, but a decrease in MAPK activation was repeatedly observed at 60 min after treatment with the *S.* Typhimurium *fliC fljB* mutant (Supplemental Figure 6).

Next, we examined whether *S.* Typhimurium-induced PTI-associated gene reprogramming was also compromised in *fls2* mutant seedlings. To this end, we inoculated Col-0 and *fls2* seedlings with *S.* Typhimurium wild-type and the *prgH* mutant for 30 min and 2 h and tested the expression of PTI marker genes by qPCR. We found that the absence of a functional FLS2 reduced gene induction by wild-type and *prgH* treatments (Figure 6). Altogether, these results indicate that FLS2-mediated flagellin recognition is an important component in *S.* Typhimurium activation of downstream responses. Furthermore, we concluded that *S.* Typhimurium possesses PAMPs other than flagellin that are perceived in *Arabidopsis*.

S. enterica Strains Carry Divergent flg22 Sequences with Reduced PTI-Inducing Activities

S. Senftenberg strains carrying the O antigen 1,3,19 were recently reported to induce chlorosis and wilting of *Arabidopsis* leaves (Berger et al., 2011). The development of the disease-like symptoms was shown to be independent of FLS2 and the authors proposed that the O antigen 1,3,19 could be a new *Salmonella* PAMP recognized in plants (Berger et al., 2011). We therefore wanted to assess whether *S.* Senftenberg strains carrying the O antigen 1,3,19 were also recognized via its flagellin. We obtained the *S.* Senftenberg strain 20070885, responsible for a salmonellosis outbreak in the UK and characterized in previous studies (Berger et al., 2009, 2011), and we used it to test MAPK activation and defense gene expression in Col-0 and *fls2* plants. Similarly to our results with *S.* Typhimurium, we observed MAPK activation and the induction of several PTI marker genes in response to *S.* Senftenberg in Col-0 and a reduced response in the *fls2* mutant (Figures 5C and 6). Interestingly, inoculation with *S.* Senftenberg induced stronger responses than inoculation with *S.* Typhimurium both in Col-0 and *fls2*. This suggests that there are PAMPs in *S.* Senftenberg, other than the FLS2-recognized epitope, which are stronger elicitors of PTI in *Arabidopsis* than those of *S.* Typhimurium. Since the *S.* Senftenberg-induced responses were reduced in *fls2* seedlings, we concluded that besides the proposed recognition of the O antigen 1,3,19, *S.* Senftenberg flagellin is recognized by FLS2. These data provide further evidence that FLS2 is involved in the recognition of different *S. enterica* serovars.

GenBank searches for *S.* Senftenberg *fliC*-encoding genes revealed two sequences that differed only in a stretch of five amino acids. Interestingly, those five amino acid changes are located in the flg22 epitope (Felix et al., 1999). The amino acid changes lead to an flg22 peptide (hereafter referred to as flg22-SS) that is more divergent from flg22 than the flg22-ST peptide (Figure 2). Two of the sequences recovered from the GenBank corresponded to the recently sequenced *S.* Senftenberg strain SS209 (Grepinet et al., 2012) and the strain SGSC2516 from the Calgary stock center (Mortimer et al., 2004); these two sequences were 100% identical and carried the same flg22 sequence as *S.* Typhimurium (flg22-ST). Furthermore, we found in the GenBank the *fliC* sequences

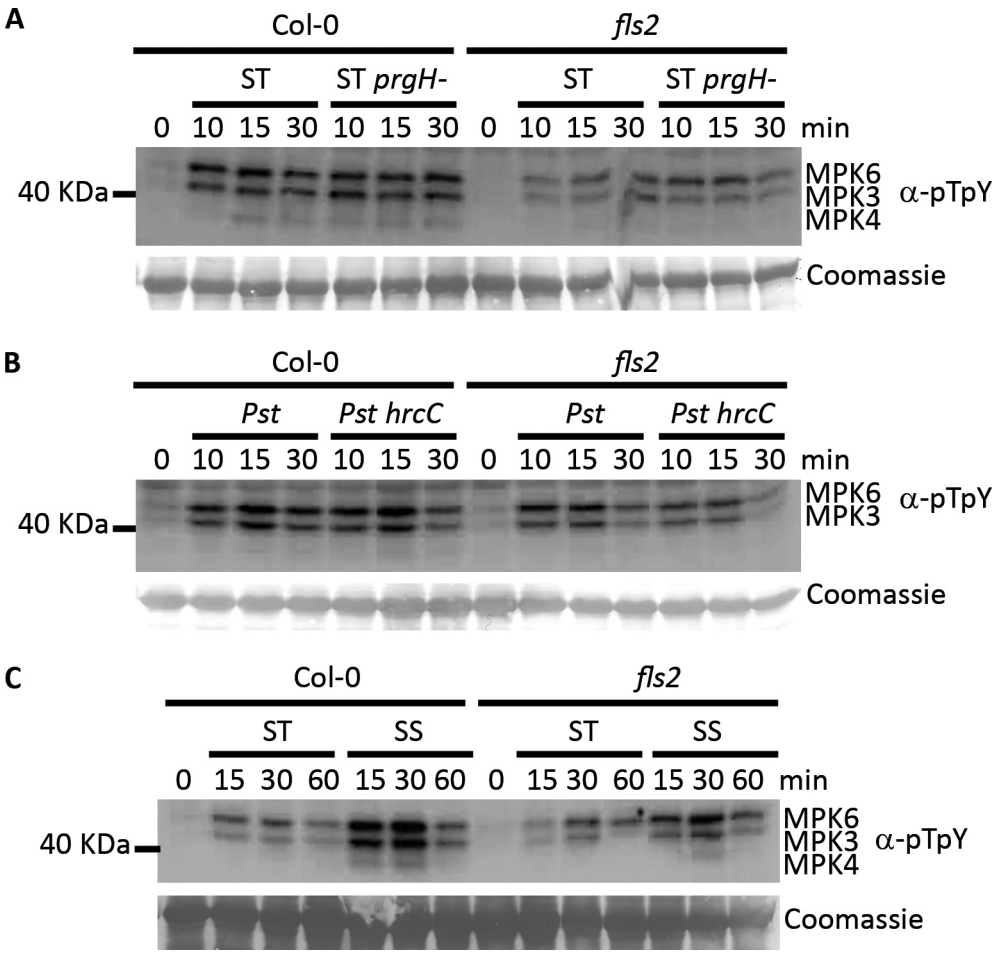

Figure 5. MAPK activation in *Arabidopsis* seedlings in response to *S. enterica* and *P. syringae* treatments.

MAPK activation in Col-0 and *fls2 in* response to *S.* Typhimurium wild-type (ST) and *S.* Typhimurium *prgH*$^{-}$ (ST *prgH*$^{-}$) **(A)**, in response to *Pst* DC3000 (*Pst*) and *Pst* DC3000 *hrcC* (*Pst hrcC*) **(B)**, and in response to *S.* Typhimurium wild-type (ST) and *S.* Senftenberg (SS) **(C)**. *Arabidopsis* seedlings in MS plates with low agar concentration were treated with the bacterial solutions at 1 × 10^{8} cfu ml^{-1} for the indicated times.

from three other *S.* Senftenberg strains: S08766–03 isolated from turkey in Austria; S05219–03 and S06320–03 isolated from chicken in the UK (Tankouo-Sandjong et al., 2008), which were 100% identical to each other and carried the *S.* Senftenberg specific flg22-SS sequence. Interestingly, these two groups of *fliC* sequences presented almost 100% identity at both the amino acid and nucleotide levels throughout the entire sequence except for variations located in the flg22 domain recognized by plants (Supplemental Figures 7 and 8). To determine which *fliC* sequence is present in the *S.* Senftenberg strain 20070885 tested in *Arabidopsis*, we amplified and sequenced the N-terminal region of the *fliC* gene. The sequence obtained showed almost 100% identity with the *fliC* variant from the *S.* Senftenberg strain SGSC2516 and indicated that the *S.* Senftenberg 20070885 strain carries the flg22-ST sequence (Supplemental Figures 7 and 8). Supplemental Figure 7 shows the protein alignment of the deduced sequence from *S.* Senftenberg strain 20070885, with one representative sequence of each FliC variant of *S.* Senftenberg and the FliC sequence of *S.* Typhimurium 14028s. Supplemental Figure 8 presents the nucleotide alignment of the sequence retrieved from *S.* Senftenberg strain 20070885, with one representative sequence of each *fliC* variant of *S.* Senftenberg, and shows that nucleotide changes are concentrated in the flg22-coding region.

We were intrigued by the occurrence of the flg22-SS sequence and compared it with the flg22 domain of other enterobacteria. We observed that, whereas the tyrosine at position 7 is found in other species, the LYCVK motif appears specific to some *S.* Senftenberg strains (Figure 3A). We synthesized the flg22-SS peptide and compared its activity with that of flg22 and flg22-ST in PTI assays using *Arabidopsis* seedlings. The flg22-SS peptide showed reduced activity in seedling growth arrest, MAPK activation, and ROS production, and showed a minor reduction in the induction of PTI marker genes (Figures 3 and 4). Since *Brassicaceous* and *Solanaceous* plants present differences in their flagellin recognition specificities (Robatzek et al., 2007; Clarke et al., 2013), we decided

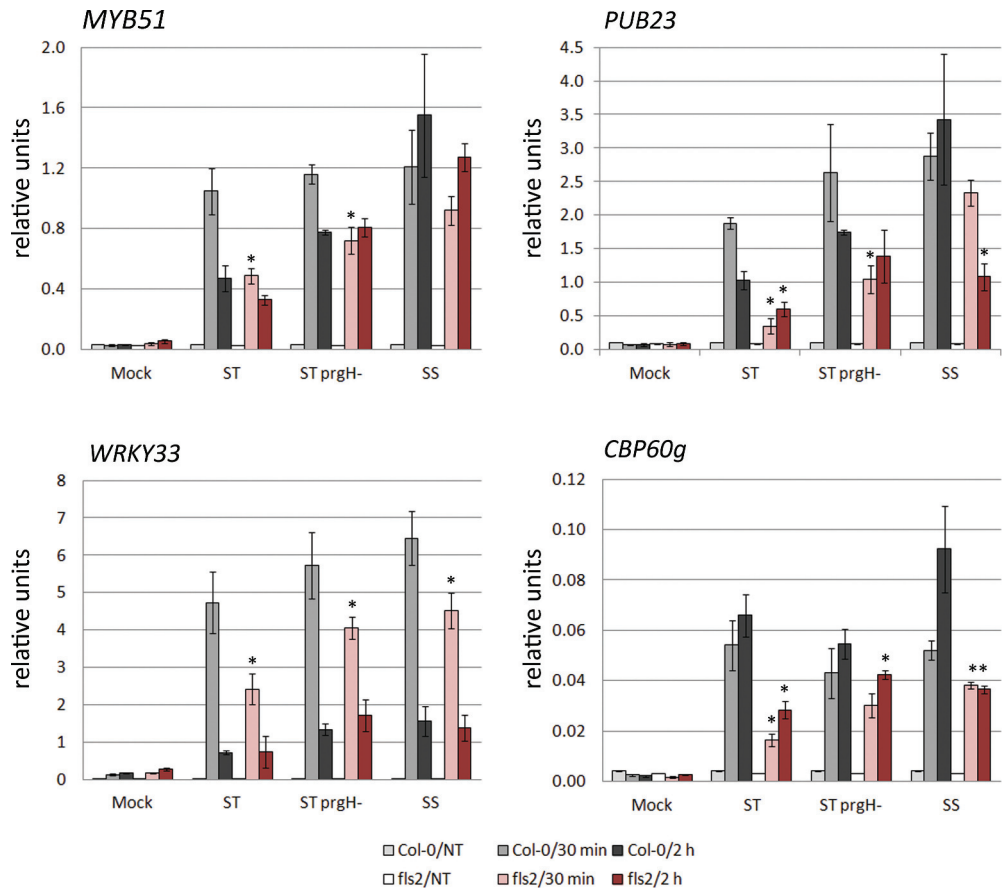

Figure 6. PTI Marker Gene Induction in Col-0 and *fls2* Seedlings at 30 min and 2 h in Response to *S.* Typhimurium Wild-Type (ST), *S.* Typhimurium *prgH*$^{-}$ (ST *prgH*$^{-}$), and *S.* Senftenberg (SS) Bacteria.

Transcript accumulation is expressed relative to the average of the transcript level of two reference genes. Bars represent means of three replicates ± SD. Results are representative of three independent experiments. Stars indicate significant differences between Col-0 and *fls2* at the indicated time point (*t*-test, *p*-value < 0.05).

to test whether the flg22-SS sequence could be recognized in *Nicotiana benthamiana* and tomato (*S. lycopersicum* cv. *Money-Maker*). Similarly to that observed in *Arabidopsis*, the flg22-SS peptide was inactive in *N. benthamiana* leaf discs whereas flg22 and flg22-ST triggered a similar ROS burst (Supplemental Figure 9A). Interestingly, when using tomato leaf discs, the flg22 and flg22-ST peptides induced a strong ROS burst and the flg22-SS induced reduced but detectable ROS production (Supplemental Figure 9B). The observed variation in the peptide activity depending on the assays may reflect different thresholds of receptor activation for the induction of the responses. In summary, these results indicate that *S. enterica* strains are variably recognized via its flagellins in different plant species.

DISCUSSION

In order to gain information about the interaction between *Arabidopsis* and *S. enterica*, we performed a genome-wide transcriptome analysis of *Arabidopsis* responses to *S.* Typhimurium wild-type and the T3SS mutant *prgH* at different time points after inoculation (2, 4, 6, 12, and 24 h). We observed that the *prgH* mutant leads to a stronger transcriptional reprogramming than wild-type, which was especially evident at the latest time points of the analysis. Analysis of the GO term enrichment and marker genes for different hormone and immunity pathways showed that the induced genes are enriched in stress response markers. In sum, this transcriptional analysis led us to conclude that *S.* Typhimurium induces various stress-signaling pathways in *Arabidopsis*, which are reduced by *S.* Typhimurium wild-type at later time points whereas they stay up-regulated in *prgH*-challenged plants. These results are in agreement with our previous study showing enhanced expression of defense genes at 24 h after inoculation with the *prgH* mutant (Schikora et al., 2011). When looking at the repressed genes, we observed enrichment in GO terms related to electron transport and photosynthesis and interestingly, microarray analysis showed the repression of several chloroplastic genes at 6 h post inoculation with both bacteria. All these observations suggest that

Arabidopsis defenses to *S.* Typhimurium include the repression of photosynthetic processes, possibly to redistribute its energy to defenses and avoid oxidative stress. Previous reports showing that photosynthesis decreases in response to microbial infections and PAMP treatments are in favor of this hypothesis (Bonfig et al., 2006; Gohre et al., 2011).

Bacterial strains with defects in the T3SS, such as the *Pst* DC3000 *hrcC* mutant, induce responses that are very similar to those triggered by purified PAMPs (Thilmony et al., 2006; Tsuda et al., 2008), probably because the T3SS effectors that interfere with PTI cannot be delivered into the plant cells. We compared the transcriptional reprogramming induced by *prgH* at early time points with that caused by PAMPs or PTI-inducing bacteria and showed that *S.* Typhimurium triggers a transcriptional reprogramming similar to that occurring during PTI. We therefore wondered about the nature of the *S. enterica* PAMP and the *Arabidopsis* receptor that activates this transcriptional response. We showed that the flg22 epitope from *S.* Typhimurium and other enterobacteria, which we called flg22-ST, is recognized in *Arabidopsis* by FLS2. This recognition event initiates various PTI hallmarks, such as growth arrest, ROS accumulation, MAPK activation, defense gene induction, and enhanced resistance to *Pst* DC3000. While preparing this manuscript, Meng and co-workers (2013) showed that the *fliC*-encoded flagellin and the derived flg22-ST peptide (called Seflg22 in their study) are the main *S.* Typhimurium PAMPs in tomato and *N. benthamiana*. *fliC* is the most widely conserved flagellin-encoding gene and is present in all *Salmonellae* (Macnab, 1992). Given that divergent flg22 sequences exist in *S. enterica* serovars, we decided to keep our nomenclature and name flg22-ST and flg22-SS the peptides of *S.* Typhimurium and *S.* Senftenberg reported in this study. The flg22-ST peptide displayed a reduced activity in some PTI assays in *Arabidopsis*, such as ROS accumulation and growth inhibition, as compared with the canonical flg22. In agreement with this, the *E. coli* flg15 peptide variant (which corresponds to the flg22-ST sequence) showed 30% of the biological activity of flg22 in an alkalinization assay (Felix et al., 1999) and *S.* Typhimurium-elicited ROS accumulation in tobacco leaves was lower than that caused by *P. syringae* (Shirron and Yaron, 2011). On the contrary, flg22-ST showed a similar activity to flg22 in *N. benthamiana* and tomato (Meng et al., 2013 and this study). The differences in the results could reflect differences in the sensitivities of the assays and in the flagellin perception specificities of different plant species. For instance, tomato plants were shown to better recognize the flg15 peptide from *E. coli* than *N. benthamiana* and *Arabidopsis* (Robatzek et al., 2007), which could explain the enhanced responsiveness of tomato to the flg22-ST peptide.

Interestingly, we found sequence variations in the flg22 epitope among strains from the *S. enterica* serovar Senftenberg, resulting in flg22 peptides that are less active in immune activation in *Arabidopsis*. The fact that the *S.* Senftenberg FliC proteins differ only in five amino acids within the flg22 epitope while the remaining amino acid sequences show 100% identity suggests that the *fliC*-encoding gene from *S. enterica* is under selective pressure to avoid detection by the plant immune system. Interestingly, recent studies of *P. syringae* isolates identified non-synonymous mutations in the *fliC* gene which reduced its defense-inducing activity (Cai et al., 2011; Clarke et al., 2013). Remarkably, one of these mutations was located within the flg22 epitope in one of the five residues modified in flg22-SS. The same study also identified a second flagellin region, named flgII-28, which is also able to induce PTI in solanaceous plants (Cai et al., 2011; Clarke et al., 2013). *S. enterica* and *P. syringae* are not the only bacteria showing intra-species variation in the flg22 sequence, as it was previously reported that strains from the phytopathogen *Xanthomonas campestris* pv. *campestris* carried divergent flg22 peptide sequences that also differed in immune activation (Sun et al., 2006). Taken together, this and previous results show that PAMPs are more variable than generally assumed and that they are probably under selective pressure to avoid plant immunity (Cai et al., 2011; Clarke et al., 2013).

In order to assess the importance of flagellin recognition in the interaction between *Arabidopsis* and *S. enterica*, we tested the responses to *S. enterica* in the *Arabidopsis fls2* mutant. We found that *fls2* seedlings show compromised, though not abolished, MAPK activation and defense gene induction in response to two *S. enterica* serovars. This observation is in agreement with previous studies suggesting that flagellin perception affects PAMP-triggered transcriptional changes in a quantitative manner (Thilmony et al., 2006) and with reports suggesting that the O antigen could be another PAMP from *S. enterica* recognized in *Arabidopsis* (Berger et al., 2011). *S.* Typhimurium was reported to enter into lettuce leaves via stomata, where FLS2-mediated stomatal closure constitutes an important defense barrier (Kroupitski et al., 2009; Zeng and He, 2010). Therefore, FLS2-mediated stomatal immunity might be an important protective layer towards *S. enterica* colonization, as shown for *P. syringae* (Melotto et al., 2006; Zeng and He, 2010). It was recently reported that *S.* Typhimurium induces lower defense responses than *E. coli*, including reduced stomatal closure (Roy et al., 2013). Interestingly, both bacteria carry the same flg22 sequence and should activate FLS2-mediated stomatal closure to a similar extent. Therefore, the differences in immune activation could be either due to the ability of *S.* Typhimurium to use effectors that suppress plant immune responses or, alternatively, to the presence of other *E. coli*-originated PAMPs that are better recognized than those from *S.* Typhimurium.

The results of this study indicate that *Arabidopsis* is able to recognize the flagellin and probably other PAMPs from *S. enterica* and thereby initiate PTI. Furthermore, *S. enterica* appears to use different strategies to accomplish plant colonization and persistence either by lowering or evading PTI activation. On one hand, we observed variation in the flg22 epitope among *S. enterica* strains, suggesting that *S. enterica*

has evolved PAMPs with reduced PTI-inducing activity to evade recognition by the plant immune system. In a subsequent layer, *S. enterica* strains that activate PTI may deploy effectors to diminish the activated plant defense responses and allow endophytic colonization. Interestingly, we showed that the SPI-1 gene *prgH* is expressed in contact with *Arabidopsis* seedlings and recent reports suggest that *S. enterica* effectors can suppress plant immunity and be recognized in plants (Schikora et al., 2011; Shirron and Yaron 2011; Ustun et al., 2011), but the *S. enterica*-mediated delivery of effectors to plant cells has not yet been demonstrated. In conclusion, the finding that PTI is activated in plants by human enteropathogenic bacteria suggests that it may constitute an effective barrier to limit endophytic colonization and it can be exploited to find new strategies to reduce the number of salmonellosis outbreaks.

METHODS

Plant and Bacterial Growth Conditions

S. Typhimurium 14028s wild-type and the *prgH* mutant were described elsewhere (Schikora et al., 2011); the *prgH* mutant is *prgH*020::Tn*5lacZY* (Tet^r) (Beuzon et al., 2001). The *S.* Typhimurium strain SL1344 carrying single-copy chromosomal insertions of P*prgH-gfp*+ P*rpsM-gfp*+ are, respectively, JH3010 and JH3016 as described (Hautefort et al., 2003). The *S.* Typhimurium flagellin mutant and respective wild-type were the following: SL3201 *fliC*::Tn10 *fljB*::MudJ (Schmitt et al., 2001). The *S.* Senftenberg strain 20070885 (Berger et al., 2011) was used and was serotyped for O antigen using commercial antisera (BIO-RAD). *Salmonella* strains were cultured in LB broth, supplemented with antibiotics if appropriate, at 37°C with agitation. Bacteria were harvested by centrifugation at 3800 r.p.m. and re-suspended in 10 mM $MgCl_2$. *P. syringae* pv. *tomato* (*Pst*) DC3000 wild-type and *hrcC* mutant were grown in NYGA plates supplemented with Rifampicin 50 µg ml^{-1}, at 28°C for 24 h and re-suspended in 10 mM $MgCl_2$. *Arabidopsis thaliana* Col-0 and the *fls2* mutant SALK_093905 were used. For *in vitro* assays, *Arabidopsis* seedlings were grown on plates with MS medium and low agar concentration (half-strength Murashige and Skoog (Sigma #M6899), 1% sucrose, 0.5% agar, 0.5% MES, pH 5.7) in long-day conditions (16 h light, 22°C). Thirteen days after sowing, seedlings were transferred to liquid MS (half-strength Murashige and Skoog (Sigma #M6899), 0.5% MES, pH 5.7) and placed overnight in long-day conditions. Fourteen-day-old seedlings in liquid MS were inoculated for RNA extractions, *S.* Typhimurium growth assays, and microscopy analyses. For *Pst* DC3000 growth assays and ROS production, *Arabidopsis* plants were grown on soil in short-day conditions (8 h light, 22°C) and *N. benthamiana* and tomato (*S. lycopersicum* cv. *Money-Maker*) plants were grown on soil in long-day conditions.

S. enterica Inoculations and Growth Assays

All *S. enterica* inoculations were performed at a final concentration of 2 × 10^8 cfu ml^{-1}, in long-day conditions (16 h light, 22°C). Seedlings were inoculated with *S. enterica* by adding equal volumes of bacterial solution or mock to the liquid MS. For growth assays, inoculated seedlings were sampled at the indicated time points and serial dilutions of the homogenates were plated on LB agar plates with the corresponding antibiotics and incubated at 37°C.

RNA Extractions and qPCR

For RNA extractions, seedlings were inoculated with identical volumes of mock (10 mM $MgCl_2$) or bacterial solutions. For each point, RNA samples were prepared by pooling three to five entire seedlings at stage 1.04 (Principal growth stage 1, according to Boyes et al. (2001)). Seedlings were harvested at the indicated times and frozen in liquid nitrogen. Total RNA extractions were performed using the RNeasy plant minikit (Qiagen) and on-column DNase treatment was performed using the RNase-Free DNase Set (Qiagen) following the manufacturer's recommendations. RNA quantification and quality were measured in a NanoDrop (Thermo Scientific). For qPCR analyses, cDNAs were synthesized from 1 µg total RNA using oligo(dT) and SuperScript III reverse transcriptase (Invitrogen) in a 30-µl total volume. For the synthesis of cDNA, RNAs were heated at 65°C for 10 min, RT mix was added, and the reaction proceeded at 42°C for 1 h. cDNAs were then heated at 75°C for 15 min and stored at 4°C. qPCR reactions were performed in 10 µl final volume with 1 µl RT reaction, 100 nM final concentration of each primer pair, and SYBR FAST Universal qPCR kit (Kapa Biosystems). *AT5G06110* and *AT4G24820* were used as reference genes. Primer sequences are available upon request. All reactions were performed in a CFX384 Touch™ Real-Time PCR Detection System (BIO-RAD) as follows: 95°C for 30 s, 40 × 95°C for 5 s, and 60°C for 20 s; and a dissociation step to discriminate primer dimers and PCR products. Three replicate PCRs were performed for each cDNA in every run.

Transcriptome Studies

The microarray analysis was carried out at the Unité de Recherche en Génomique Végétale (URGV, Evry, France), using the CATMAv5 arrays containing 31 776 gene-specific tags corresponding to 22 089 genes from *Arabidopsis* (Crowe et al., 2003; Hilson et al., 2004). For each comparison, one technical replicate with fluorochrome reversal (dye-swap) was performed for each biological replicate (i.e. four hybridizations per comparison). The labeling of cRNAs with Cy3-dUTP or Cy5-dUTP (Perkin-Elmer-NEN Life Science Products), the hybridization to the slides, and the scanning were performed as previously described (Lurin et al., 2004).

Statistical Analysis of Microarray Data

Experiments were designed with the statistics group of the URGV. For each array, the raw data comprised the logarithm of median feature pixel intensity at wavelengths 635 nm (red) and 532 nm (green) and no background was subtracted. An array-by-array normalization was performed to remove

systematic biases. First, spots considered badly formed features were excluded. Then, a global intensity-dependent normalization using the LOESS procedure was performed to correct the dye bias. Finally, for each block, the log-ratio median calculated over the values for the entire block was subtracted from each individual log-ratio value to correct print tip effects. Differential analysis was based on the log ratios averaged on the dye-swap: the technical replicates were averaged to get one log ratio per biological replicate and these values were used to perform a paired *t*-test. A trimmed variance is calculated from spots which do not display extreme variance (see details in Gagnot et al., (2008)). The raw *P*-values were adjusted by the Bonferroni method, which controls the Family Wise Error Rate in order to keep a strong control of the false positives in a multiple-comparison context. We considered as being differentially expressed the probes with a Bonferroni *P*-value < 0.05.

Data Deposition

Microarray data from this article were deposited at Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo/, accession no. GSE38828) and at CATdb (http://urgv.evry.inra.fr/CATdb/, Project: RA11-01_prgH) according to the 'Minimum Information About a Microarray Experiment' standards.

PTI Assays

The flg22 peptide was bought from GeneCust Europe; the flg22-ST and flg22-SS peptides were bought from PROTEOGENIX SAS with >80% purity and re-suspended in sterile ultrapure H_2O. ROS production was quantified as previously described (Gomez-Gomez and Boller, 2000). Briefly, 6-mm leaf discs of 5-week-old *Arabidopsis* plants or 5-mm leaf discs of 6-week-old *N. benthamiana* or tomato plants were placed in 96-well plates with 200 µl sterile H_2O and incubated overnight at room temperature. Sixteen hours later, the water was replaced with a solution containing 100 nM flagellin peptide, 17 µg ml^{-1} luminol (SIGMA), and 10 µg ml^{-1} horseradish peroxidase (SIGMA). Luminescence was detected using a TECAN infinite M200. For growth inhibition assays, seedlings were grown in solid MS plates, transferred to liquid MS with or without peptide at day 5, and weighted 7 d after. Induced resistance to *Pst* DC3000 was performed by using a needle-less syringe to infiltrate 1 µM peptide to the leaf abaxial side and a bacterial solution at 1×10^5 cfu ml^{-1} 24 h after, as described by Zipfel et al. (2004). Bacterial titers were determined at day 2 as previously described (Garcia et al., 2010). Callose was detected using aniline blue staining as previously described (Daudi et al., 2012); three rosette leaves from 4–5-week-old plants and five plants per sample were used in two independent biological replicates. Phytohormone quantification was performed as previously described (Leitner et al., 2008).

Microscopy

Callose deposits were visualized with a Leica MZ16F fluorescence microscope and a Leica 10447354 filter. GFP signals were monitored with a confocal microscope (TCS SP2-AOBS; Leica).

Protein Extraction and Western Blot Analysis

For the MAPK activation assays, seedlings were grown on solid MS, treated with MS alone on day 13 after sowing, and treated with liquid MS alone (mock) or supplemented with peptide or bacteria at the given concentrations on day 14. For peptide treatments, proteins were extracted using 2 SDS–PAGE loading buffer and denatured at 95°C for 10 min. Protein quantification for normalization was performed using an Amido Black assay. For bacterial treatments, proteins were extracted using a native buffer containing 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% NP40, 5 mM EGTA, 0.1 mM DTT, 1 Complete protease inhibitors (Roche Applied Science), 1 mM NaF, 0.5 mM Na_3VO_4, 15 mM β-Glycerophosphate, 15 mM 4-Nitrophenyl phosphate, and protein quantification for normalization was performed using a Bradford assay. Chemicals were purchased from SIGMA unless otherwise stated. Protein samples were separated on 10% acrylamide/bis-acrylamide (SIGMA) SDS–PAGE gels and transferred on 0.45-µm PVDF membranes (GE Healthcare). Blocking was performed in TBST containing 5% BSA, blots were incubated overnight with anti-pTpY antibody (rabbit anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204); Cell Signaling), washed in TBST, and incubated with the goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (SIGMA) diluted to 1/20 000. The chemiluminescent detection of HRP was performed with ECL reagent (GE Healthcare) and an imaging system (GeneGnome, Syngene). After immunoblotting and imaging, the blots were stained with a solution of Coomassie blue for protein visualization: 0.75% Coomassie Brilliant Blue R-250 (821616, ICN Biomedicals Inc.), 20% ethanol, 10% acetic acid.

Flagellin Sequences and Alignments

Genomic DNA from *S. enterica* serovar Senftenberg strain 20070885 was isolated using an UltraClean Microbial DNA Isolation kit (MO BIO Laboratories Inc., California) following the manufacturer's recommendations. A 428-nt fragment of the N-terminal region of the *fliC* gene was amplified using the following primers: FlgSS-F TGAACAAATCTCAGTCCTCACTGAGTTCCGC and FlgSS-R CAGATCGATGGTAATGGTTTCACCATCG. PCR reaction was performed in 20 µl using 250-nM primers, 500-µM dNTPs, and Taq DNA Polymerase with ThermopPol buffer (New England BioLabs). The PCR reaction was the following: 94°C for 5 min; 30 cycles of 94°C for 30 s/54°C for 45 s/72°C for 1 min, and 72°C for 5 min. Multiple sequence alignments were performed using Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo/) or Multalin (http://multalin.toulouse.inra.fr/multalin/). Accession numbers of the *fliC* sequences used in this study: *P. aeruginosa* ATCC 700888: Accession ZP_15627443; *P. syringae* pv. *tomato* str. DC3000: Accession

NP_791772; *S.* Typhimurium 14028S: Accession ACY88831; *S.* Senftenberg S05219 03: Accession ABR18492; *S.* Senftenberg SGSC2516: Accession AY649714; *Yersinia rohdei* ATCC 43380: Accession ZP_04614654; *Shigella dysenteriae* 1617: Accession ZP_07683944; *E. coli*: Accession CAJ90397; *Providencia sneebia* DSM 19967: Accession ZP_16368948; *Brenneria* sp. EniD312: Accession ZP_09016061; *Pectobacterium wasabiae* CFBP 3304: Accession ZP_15541554; *Pectobacterium carotovorum* subsp. *carotovorum*: Accession AFR46596; *Dickeya dadantii* 3937: Accession YP_003883600.

SUPPLEMENTARY DATA

Supplementary Data are available at *Molecular Plant Online.*

FUNDING

This work was supported by the European Research Area Network (ERA-Net) through the ERASysBio PLUS project SHIPREC.

ACKNOWLEDGMENTS

We thank David Holden (Imperial Collage, UK) for the *S.* Typhimurium 14028 wild-type and *prgH* mutant; Mark Jepson (Bristol University, UK) for *S.* Typhimurium P*rpsM-gfp+* and P*prgH-gfp+*; Nicola Holden (James Hutton Institute, UK) for the *S.* Senftenberg strain 20070885; Allison O'Brien for the *S.* Typhimurium *fliCfljB* double mutant; Andeol Falcon for the *N. benthamiana* plants; Fabien Marcel for the tomato plants; and Jean Colcombet and Axel de Zelicourt (URGV) for helpful discussions. No conflict of interest declared.

REFERENCES

Aalto, M.K., Helenius, E., Kariola, T., Pennanen, V., Heino, P., Horak, H., Puzorjova, I., Kollist, H., and Palva, E.T. (2012). ERD15—an attenuator of plant ABA responses and stomatal aperture. Plant Sci. **182**, 19–28.

Asai, T., Tena, G., Plotnikova, J., Willmann, M.R., Chiu, W.L., Gomez-Gomez, L., Boller, T., Ausubel, F.M., and Sheen, J. (2002). MAP kinase signalling cascade in *Arabidopsis* innate immunity. Nature. **415**, 977–983.

Barak, J.D., and Schroeder, B.K. (2012). Interrelationships of food safety and plant pathology: the life cycle of human pathogens on plants. Annu. Rev. Phytopathol. **50**, 241–266.

Barak, J.D., Kramer, L.C., and Hao, L.Y. (2011). Colonization of tomato plants by *Salmonella enterica* is cultivar dependent, and type 1 trichomes are preferred colonization sites. Appl. Environ. Microbiol. **77**, 498–504.

Behlau, I., and Miller, S.I. (1993). A PhoP-repressed gene promotes *Salmonella* typhimurium invasion of epithelial cells. J. Bacteriol. **175**, 4475–4484.

Berger, C.N., Brown, D.J., Shaw, R.K., Minuzzi, F., Feys, B., and Frankel, G. (2011). *Salmonella enterica* strains belonging to O serogroup 1,3,19 induce chlorosis and wilting of *Arabidopsis thaliana* leaves. Environ. Microbiol. **13**, 1299–1308.

Berger, C.N., Shaw, R.K., Brown, D.J., Mather, H., Clare, S., Dougan, G., Pallen, M.J., and Frankel, G. (2009). Interaction of *Salmonella enterica* with basil and other salad leaves. ISME J. **3**, 261–265.

Beuzon, C.R.K., Unsworth, E., and Holden, D.W. (2001). *In vivo* genetic analysis indicates that PhoP–PhoQ and the Salmonella pathogenicity island 2 type III secretion system contribute independently to *Salmonella enterica* serovar Typhimurium virulence. Infect. Immun. **69**, 7254–7261.

Birkenbihl, R.P., Diezel, C., and Somssich, I.E. (2012). *Arabidopsis* WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward *Botrytis cinerea* infection. Plant Physiol. **159**, 266–285.

Bonfig, K.B., Schreiber, U., Gabler, A., Roitsch, T., and Berger, S. (2006). Infection with virulent and avirulent *P. syringae* strains differentially affects photosynthesis and sink metabolism in *Arabidopsis* leaves. Planta. **225**, 1–12.

Boudsocq, M., Willmann, M.R., McCormack, M., Lee, H., Shan, L., He, P., Bush, J., Cheng, S.H., and Sheen, J. (2010). Differential innate immune signalling via Ca(2+) sensor protein kinases. Nature. **464**, 418–422.

Boyes, D.C., Zayed, A.M., Ascenzi, R., McCaskill, A.J., Hoffman, N.E., Davis, K.R., and Gorlach, J. (2001). Growth stage-based phenotypic analysis of *Arabidopsis*: a model for high throughput functional genomics in plants. Plant Cell. **13**, 1499–1510.

Broz, P., Ohlson, M.B., and Monack, D.M. (2012). Innate immune response to *Salmonella* typhimurium, a model enteric pathogen. Gut Microbes. **3**, 62–70.

Bruno, V.M., Hannemann, S., Lara-Tejero, M., Flavell, R.A., Kleinstein, S.H., and Galan, J.E. (2009). *Salmonella* Typhimurium type III secretion effectors stimulate innate immune responses in cultured epithelial cells. PLoS Pathog. **5**, e1000538.

Cai, R., Lewis, J., Yan, S., Liu, H., Clarke, C.R., Campanile, F., Almeida, N.F., Studholme, D.J., 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.

Clarke, C.R., Chinchilla, D., Hind, S.R., Taguchi, F., Miki, R., Ichinose, T., Martin, G.B., Leman, S., Felix, G., and Vinatzer, B.A. (2013). Allelic variation in two distinct *Pseudomonas syringae* flagellin epitopes modulates the strength of plant immune responses but not bacterial motility. New Phytol. **200**, 847–860

Clay, N.K., Adio, A.M., Denoux, C., Jander, G., and Ausubel, F.M. (2009). Glucosinolate metabolites required for an *Arabidopsis* innate immune response. Science. **323**, 95–101.

Cooley, M.B., Miller, W.G., and Mandrell, R.E. (2003). Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. Appl. Environ. Microbiol. **69**, 4915–4926.

Crowe, M.L., Serizet, C., Thareau, V., Aubourg, S., Rouze, P., Hilson, P., Beynon, J., Weisbeek, P., van Hummelen, P., Reymond, P., et al. (2003). CATMA: a complete *Arabidopsis* GST database. Nucleic Acids Res. **31**, 156–158.

Daudi, A., Cheng, Z., O'Brien, J.A., Mammarella, N., Khan, S., Ausubel, F.M., and Bolwell, G.P. (2012). The apoplastic oxidative

burst peroxidase in *Arabidopsis* is a major component of pattern-triggered immunity. Plant Cell. **24**, 275–287.

Felix, G., Duran, J.D., Volko, S., and Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J. **18**, 265–276.

Feys, B.J., Moisan, L.J., Newman, M.A., and Parker, J.E. (2001). Direct interaction between the *Arabidopsis* disease resistance signaling proteins, EDS1 and PAD4. EMBO J. **20**, 5400–5411.

Gagnot, S., Tamby, J.P., Martin-Magniette, M.L., Bitton, F., Taconnat, L., Balzergue, S., Aubourg, S., Renou, J.P., Lecharny, A., and Brunaud, V. (2008). CATdb: a public access to *Arabidopsis* transcriptome data from the URGV-CATMA platform. Nucleic Acids Res. **36**, D986–D990.

Galan, J.E. (2009). Common themes in the design and function of bacterial effectors. Cell Host Microbe. **5**, 571–579.

Garcia, A.V., Blanvillain-Baufume, S., Huibers, R.P., Wiermer, M., Li, G., Gobbato, E., Rietz, S., and Parker, J.E. (2010). Balanced nuclear and cytoplasmic activities of EDS1 are required for a complete plant innate immune response. PLoS Pathog. **6**, e1000970.

Gohre, V., Jones, A.M., Sklenar, J., Robatzek, S., and Weber, A.P. (2011). Molecular crosstalk between PAMP-triggered immunity and photosynthesis. Mol. Plant Microbe Interact. **25**, 1083–1092.

Golberg, D., Kroupitski, Y., Belausov, E., Pinto, R. and Sela, S. (2011). *Salmonella Typhimurium* internalization is variable in leafy vegetables and fresh herbs. Int. J. Food Microbiol. **145**, 250–257.

Gomez-Gomez, L., and 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.

Gomez-Gomez, L., Felix, G., and Boller, T. (1999). A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. Plant J. **18**, 277–284.

Goudeau, D.M., Parker, C.T., Zhou, Y., Sela, S., Kroupitski, Y., and Brandl, M.T. (2013). The salmonella transcriptome in lettuce and cilantro soft rot reveals a niche overlap with the animal host intestine. Appl. Environ. Microbiol. **79**, 250–262.

Grepinet, O., Boumart, Z., Virlogeux-Payant, I., Loux, V., Chiapello, H., Gendrault, A., Gibrat, J.F., Chemaly, M., and Velge, P. (2012). Genome sequence of the persistent *Salmonella enterica* subsp. *enterica* serotype Senftenberg strain SS209. J. Bacteriol. **194**, 2385–2386.

Hautefort, I., Proenca, M.J., and Hinton, J.C. (2003). Single-copy green fluorescent protein gene fusions allow accurate measurement of Salmonella gene expression *in vitro* and during infection of mammalian cells. Appl. Environ. Microbiol. **69**, 7480–7491.

Heidrich, K., Blanvillain-Baufume, S., and Parker, J.E. (2012). Molecular and spatial constraints on NB-LRR receptor signaling. Curr. Opin. Plant Biol. **15**, 385–391.

Hilson, P., Allemeersch, J., Altmann, T., Aubourg, S., Avon, A., Beynon, J., Bhalerao, R.P., Bitton, F., Caboche, M., Cannoot, B., et al. (2004). Versatile gene-specific sequence tags for *Arabidopsis* functional genomics: transcript profiling and reverse genetics applications. Genome Res. **14**, 2176–2189.

Hu, Y., Dong, Q., and Yu, D. (2012). *Arabidopsis* WRKY46 coordinates with WRKY70 and WRKY53 in basal resistance against pathogen *Pseudomonas syringae*. Plant Sci. 185–186, 288–297.

Huffaker, A., and Ryan, C.A. (2007). Endogenous peptide defense signals in *Arabidopsis* differentially amplify signaling for the innate immune response. Proc. Natl Acad. Sci. U S A. **104**, 10732–10736.

Iniguez, A.L., Dong, Y., Carter, H.D., Ahmer, B.M., Stone, J.M., and Triplett, E.W. (2005). Regulation of enteric endophytic bacterial colonization by plant defenses. Mol. Plant Microbe Interact. **18**, 169–178.

Jagadeeswaran, G., Raina, S., Acharya, B.R., Maqbool, S.B., Mosher, S.L., Appel, H.M., Schultz, J.C., Klessig, D.F., and Raina, R. (2007). *Arabidopsis* GH3-LIKE DEFENSE GENE 1 is required for accumulation of salicylic acid, activation of defense responses and resistance to *Pseudomonas syringae*. Plant J. **51**, 234–246.

Kroupitski, Y., Golberg, D., Belausov, E., Pinto, R., Swartzberg, D., Granot, D., and Sela, S. (2009). Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. Appl. Environ. Microbiol. **75**, 6076–6086.

Leitner, M., Kaiser, R., Rasmussen, M.O., Driguez, H., Boland, W., and Mithofer, A. (2008). Microbial oligosaccharides differentially induce volatiles and signalling components in *Medicago truncatula*. Phytochemistry. **69**, 2029–2040.

Li, G., Meng, X., Wang, R., Mao, G., Han, L., Liu, Y., and Zhang, S. (2012). Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in *Arabidopsis*. PLoS Genet. **8**, e1002767.

Li, J., Brader, G., and Palva, E.T. (2004). The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell. **16**, 319–331.

Logemann, E., Birkenbihl, R.P., Rawat, V., Schneeberger, K., Schmelzer, E., and Somssich, I.E. (2013). Functional dissection of the PROPEP2 and PROPEP3 promoters reveals the importance of WRKY factors in mediating microbe-associated molecular pattern-induced expression. New Phytol. **198**, 1165–1177.

Lurin, C., Andres, C., Aubourg, S., Bellaoui, M., Bitton, F., Bruyere, C., Caboche, M., Debast, C., Gualberto, J., Hoffmann, B.A. et al. (2004). Genome-wide analysis of *Arabidopsis* pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. Plant Cell. **16**, 2089–2103.

Macnab, R.M. (1992). Genetics and biogenesis of bacterial flagella. Annu. Rev. Genet. **26**, 131–158.

Melotto, M., Underwood, W., Koczan, J., Nomura, K., and He, S.Y. (2006). Plant stomata function in innate immunity against bacterial invasion. Cell. **126**, 969–980.

Meng, F., Altier, C., and Martin, G.B. (2013). Salmonella colonization activates the plant immune system and benefits from association with plant pathogenic bacteria. Environ. Microbiol. **15**, 2418–2430.

Mortimer, C.K., Peters, T.M., Gharbia, S.E., Logan, J.M., and Arnold, C. (2004). Towards the development of a DNA-sequence based approach to serotyping of *Salmonella enterica*. BMC Microbiol. **4**, 31.

Nobuta, K., Okrent, R.A., Stoutemyer, M., Rodibaugh, N., Kempema, L., Wildermuth, M.C., and Innes, R.W. (2007). The GH3 acyl adenylase family member PBS3 regulates salicylic acid-dependent defense responses in *Arabidopsis*. Plant Physiol. **144**, 1144–1156.

O'Brien, J.A., Daudi, A., Finch, P., Butt, V.S., Whitelegge, J.P., Souda, P., Ausubel, F.M., and Bolwell, G.P. (2012). A peroxidase-dependent apoplastic oxidative burst in cultured *Arabidopsis* cells functions in MAMP-elicited defense. Plant Physiol. **158**, 2013–2027.

Pawelek, J.M., Sodi, S., Chakraborty, A.K., Platt, J.T., Miller, S., Holden, D.W., Hensel, M., and Low, K.B. (2002). Salmonella pathogenicity island-2 and anticancer activity in mice. Cancer Gene Ther. **9**, 813–818.

Provart, N.J., Gil, P., Chen, W., Han, B., Chang, H.S., Wang, X., and Zhu, T. (2003). Gene expression phenotypes of *Arabidopsis* associated with sensitivity to low temperatures. Plant Physiol. **132**, 893–906.

Robatzek, S., Bittel, P., Chinchilla, D., Kochner, P., Felix, G., Shiu, S.H., and Boller, T. (2007). Molecular identification and characterization of the tomato flagellin receptor LeFLS2, an orthologue of *Arabidopsis* FLS2 exhibiting characteristically different perception specificities. Plant Mol. Biol. **64**, 539–547.

Robert-Seilaniantz, A., Grant, M., and Jones, J.D. (2011). Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu. Rev. Phytopathol. **49**, 317–343.

Roy, D., Panchal, S., Rosa, B.A., and Melotto, M. (2013). *Escherichia coli* O157:H7 induces stronger plant immunity than *Salmonella enterica* Typhimurium SL1344. Phytopathology. **103**, 326–332.

Schikora, A., Carreri, A., Charpentier, E., and Hirt, H. (2008). The dark side of the salad: *Salmonella* typhimurium overcomes the innate immune response of *Arabidopsis thaliana* and shows an endopathogenic lifestyle. PLoS One. **3**, e2279.

Schikora, A., Garcia, A.V., and Hirt, H. (2012). Plants as alternative hosts for Salmonella. Trends Plant Sci. **17**, 245–249.

Schikora, A., Virlogeux-Payant, I., Bueso, E., Garcia, A.V., Nilau, T., Charrier, A., Pelletier, S., Menanteau, P., Baccarini, M., Velge, P., et al. (2011). Conservation of Salmonella infection mechanisms in plants and animals. PLoS One. **6**, e24112.

Schmitt, C.K., Ikeda, J.S., Darnell, S.C., Watson, P.R., Bispham, J., Wallis, T.S., Weinstein, D.L., Metcalf, E.S., and O'Brien, A.D. (2001). Absence of all components of the flagellar export and synthesis machinery differentially alters virulence of *Salmonella enterica* serovar Typhimurium in models of typhoid fever, survival in macrophages, tissue culture invasiveness, and calf enterocolitis. Infect. Immun. **69**, 5619–5625.

Shirron, N., and Yaron, S. (2011). Active suppression of early immune response in tobacco by the human pathogen *Salmonella* Typhimurium. PLoS One. **6**, e18855.

Silverman, M., and Simon, M. (1980). Phase variation: genetic analysis of switching mutants. Cell. **19**, 845–854.

Straus, M.R., Rietz, S., Ver Loren van Themaat, E., Bartsch, M., and Parker, J.E. (2010). Salicylic acid antagonism of EDS1-driven cell death is important for immune and oxidative stress responses in *Arabidopsis*. Plant J. **62**, 628–640.

Sun, W., Dunning, F.M., Pfund, C., Weingarten, R., and Bent, A.F. (2006). Within-species flagellin polymorphism in *Xanthomonas campestris* pv *campestris* and its impact on elicitation of *Arabidopsis* FLAGELLIN SENSING2-dependent defenses. Plant Cell. **18**, 764–779.

Takase, T., Nakazawa, M., Ishikawa, A., Kawashima, M., Ichikawa, T., Takahashi, N., Shimada, H., Manabe, K., and Matsui, M. (2004). ydk1-D, an auxin-responsive GH3 mutant that is involved in hypocotyl and root elongation. Plant J. **37**, 471–483.

Tankouo-Sandjong, B., Sessitsch, A., Stralis-Pavese, N., Liebana, E., Kornschober, C., Allerberger, F., Hachler, H., and Bodrossy, L. (2008). Development of an oligonucleotide microarray method for Salmonella serotyping. Microb. Biotechnol. **1**, 513–522.

Thilmony, R., Underwood, W., and He, S.Y. (2006). Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and the human pathogen *Escherichia coli* O157:H7. Plant J. **46**, 34–53.

Tintor, N., Ross, A., Kanehara, K., Yamada, K., Fan, L., Kemmerling, B., Nurnberger, T., Tsuda, K., and Saijo, Y. (2013). Layered pattern receptor signaling via ethylene and endogenous elicitor peptides during *Arabidopsis* immunity to bacterial infection. Proc. Natl Acad. Sci U S A. **110**, 6211–6216.

Trujillo, M., Ichimura, K., Casais, C., and Shirasu, K. (2008). Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in *Arabidopsis*. Curr. Biol. **18**, 1396–1401.

Tsuda, K., Sato, M., Glazebrook, J., Cohen, J.D., and Katagiri, F. (2008). Interplay between MAMP-triggered and SA-mediated defense responses. Plant J. **53**, 763–775.

Ustun, S., Muller, P., Palmisano, R., Hensel, M., and Bornke, F. (2011). SseF, a type III effector protein from the mammalian pathogen *Salmonella enterica*, requires resistance-gene-mediated signalling to activate cell death in the model plant *Nicotiana benthamiana*. New Phytol. **194**, 1046–1060.

Wang, L., Tsuda, K., Sato, M., Cohen, J.D., Katagiri, F., and Glazebrook, J. (2009). *Arabidopsis* CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against *Pseudomonas syringae*. PLoS Pathog. **5**, e1000301.

Weigel, R.R., Pfitzner, U.M., and Gatz, C. (2005). Interaction of NIMIN1 with NPR1 modulates PR gene expression in *Arabidopsis*. Plant Cell. **17**, 1279–1291.

Wildermuth, M.C., Dewdney, J., Wu, G., and Ausubel, F.M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature. **414**, 562–565.

Zeng, W., and He, S.Y. (2010). A prominent role of the flagellin receptor FLAGELLIN-SENSING2 in mediating stomatal response to *Pseudomonas syringae* pv *tomato* DC3000 in *Arabidopsis*. Plant Physiol. **153**, 1188–1198.

Zipfel, C. (2009). Early molecular events in PAMP-triggered immunity. Curr. Opin. Plant Biol. **12**, 414–420.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E.J., Jones, J.D., Felix, G., and Boller, T. (2004). Bacterial disease resistance in *Arabidopsis* through flagellin perception. Nature. **428**, 764–767.