Pathogen profile

*Pseudomonas syringae* pv. *phaseolicola*: from ‘has bean’ to supermodel

DAWN L. ARNOLD¹*, HELEN C. LOVELL¹, ROBERT W. JACKSON² AND JOHN W. MANSFIELD³

¹Centre for Research in Plant Science, University of the West of England, Bristol BS16 1QY, UK
²School of Biological Sciences, University of Reading, Reading RG6 6AJ, UK
³Division of Biology, Imperial College, London SW7 2AZ, UK

**SUMMARY**

*Pseudomonas syringae* pv. *phaseolicola* causes halo blight of the common bean, *Phaseolus vulgaris*, worldwide and remains difficult to control. Races of the pathogen cause either disease symptoms or a resistant hypersensitive response on a series of differentially reacting bean cultivars. The molecular genetics of the interaction between *P. syringae* pv. *phaseolicola* and bean, and the evolution of bacterial virulence, have been investigated in depth and this research has led to important discoveries in the field of plant–microbe interactions. In this review, we discuss several of the areas of study that chart the rise of *P. syringae* pv. *phaseolicola* from a common pathogen of bean plants to a molecular plant-pathogen supermodel bacterium.

**Taxonomy**: Bacteria; Proteobacteria, gamma subdivision; order *Pseudomonadales*; family *Pseudomonadaceae*; genus *Pseudomonas*; species *Pseudomonas syringae*; Genomospecies 2; pathogenic variety *phaseolicola*.

**Microbiological properties**: Gram-negative, aerobic, motile, rod-shaped, 1.5 μm long, 0.7–1.2 μm in diameter, at least one polar flagellum, optimal temperatures for growth of 25–30 °C, oxidase negative, arginine dihydrolase negative, levan positive and elicits the hypersensitive response on tobacco.

**Host range**: Major bacterial disease of common bean (*Phaseolus vulgaris*) in temperate regions and above medium altitudes in the tropics. Natural infections have been recorded on several other legume species, including all members of the tribe *Phaseoleae* with the exception of *Desmodium* spp. and *Pisum sativum*.

**Disease symptoms**: Water-soaked lesions on leaves, pods, stems or petioles, that quickly develop greenish-yellow haloes on leaves at temperatures of less than 23 °C. Infected seeds may be symptomless, or have wrinkled or buttery-yellow patches on the seed coat. Seedling infection is recognized by general chlorosis, stunting and distortion of growth.

**Epidemiology**: Seed borne and disseminated from exudation by water-splash and wind occurring during rainfall. Bacteria invade through wounds and natural openings (notably stomata). Weedy and cultivated alternative hosts may also harbour the bacterium.

**Disease control**: Some measure of control is achieved with copper formulations and streptomycin. Pathogen-free seed and resistant cultivars are recommended.


**INTRODUCTION**

*Pseudomonas syringae* pathovar *phaseolicola* causes halo blight of the common bean, *Phaseolus vulgaris*, and the disease in leaves is classically recognized by the presence of water-soaked lesions surrounded by haloes (Fig. 1) (Murillo *et al*., 2010). Halo blight disease was characterized by Burkholder (1926) and the isolated bacterium was initially named *Phytomonas medicaginis* variant *phaseolicola*, before being renamed *Pseudomonas medicaginis* variant *phaseolicola* (Dowson, 1943) and, subsequently, *P. syringae* pathovar *phaseolicola* (Young *et al*., 1978). The final renaming grouped the bean pathogen with the many other plant-pathogenic strains also prefixed *Pseudomonas syringae*. *Pseudomonas syringae* pv. *phaseolicola* persists on dry bean seeds and becomes active after their germination (Burkholder, 1930). It can survive as both a parasite within plant tissue and as...
an epiphyte. The disease is spread within the crop by rain splash, plant-to-plant contact, irrigation water and field workers, and favours cool (less than 25 °C) moist conditions. It is prevalent worldwide, and causes most devastation in developing countries (Taylor et al., 1996a). Halo blight is considered to be such a serious disease that US law (Idaho State) demands that any crops found to contain *P. syringae* pv. *phaseolicola* must be destroyed. Up to 43% reductions in total yield have been reported and further losses occur owing to the poor quality of infected pods (Prosen et al., 1993). Halo blight is controlled mainly by sowing pathogen-free seed, the use of resistant varieties and through crop rotation (Taylor et al., 1996b).

The molecular genetics of *P. syringae* pv. *phaseolicola* have been studied extensively, leading to discoveries of general impact on our knowledge of microbial pathogenicity and plant defences. For example, the first characterization of the *hrp* genes that encode the Type III secretion system (T3SS), which delivers proteins directly into plant cells, was achieved using the halo blight bacterium. More recently, comparative analysis of the whole-genome sequences of *P. syringae* pv. *phaseolicola* and other *P. syringae* pathovars has greatly advanced our understanding of the evolution of host specificity. Here, we review the lessons learned that have led to the bean pathogen becoming a supermodel species for research on plant–pathogen interactions.

**RACE STRUCTURE AND GENE-FOR-GENE INTERACTIONS**

Bacterial pathogens manipulate their hosts by delivering effector proteins into the plant cell cytoplasm through the conserved T3SS (Casper-Lindley et al., 2002). Pioneering research by Panopolous and co-workers identified genes encoding the T3SS in *P. syringae* pv. *phaseolicola* as a region of the chromosome required for the induction of the hypersensitive reaction (HR) and pathogenicity (*hrp*) (Lindgren et al., 1986). Plants have evolved resistance proteins (*R*) that recognize a subset of effectors, termed ‘avrulcule’ (*Avr*) proteins, that trigger a defensive HR, generating an antimicrobial environment and leading to the restriction of bacterial colonization (Jones and Dangl, 2006). The gene-for-gene interaction between *Avr* and *R* proteins has led to the establishment of race structures within *P. syringae* pathovars, including *P. syringae* pv. *phaseolicola*.

Taylor et al. (1996a, b) carried out an exceptionally thorough study of *P. syringae* pv. *phaseolicola* and its host plant. They collected 893 isolates from Africa and other bean-growing areas, and used 175 to differentiate pathotypes. Nine races were identified on the basis of their interactions with eight bean cultivars and five pairs of *avr* and *R* genes (Table 1). A number of races (1, 2, 5, 6 and 7) were distributed worldwide, with race 6 predominant. Other races were found mainly in Africa: races 3 and 4 in...
East/Central Africa and races 8 and 9 in southern Africa (Taylor et al., 1996a). In total, 1048 bean accessions were also collected from Africa and the Americas, and evaluated for their resistance to *P. syringae pv. phaseolicola*. Race-specific *R* genes were detected in 49.4% of accessions with the following gene frequencies: *R1* (10.3%), *R2* (0.3%), *R3* (25.0%), *R4* (35.0%) and *R5* (0.2%) (Taylor et al., 1996b). Corresponding *avr* gene frequencies were calculated as: *AvrPphB* (27.4%), *AvrPphA* (23.9%), *AvrPphD* (23.2%), *AvrPphE* (3.5%) and *AvrPphF* (0.3%) (Taylor et al., 2003). In general, the higher the *R* gene frequency, the lower the matching *avr* gene frequency, suggesting that, were an *R* gene is widely deployed, the races of the pathogen carrying the corresponding *avr* gene are suppressed. Taylor *et al.* also found some isolates that could not be assigned to any of the nine races, results confirmed by Rico *et al.* (2003) and Rivas *et al.* (2005). Three of the predicted *P. syringae pv. phaseolicola* *avr* genes have been cloned: *AvrPphB* (A3), *AvrPphE* (A2) and *AvrPphF* (A1) (Jenner *et al.*, 1991; Mansfield *et al.*, 1994; Tsiamis *et al.*, 2000). Additional *avr* genes, *AvrPphC* and *AvrPphD*, were cloned on the basis of their contribution towards hypersensitive resistance in the nonhost plants, soybean and pea, respectively (Mansfield, 2009). *AvrPphB* triggers the HR in *Arabidopsis thaliana* accessions with the matching *RP5S* resistance gene (Shao *et al.*, 2003).

### Hyperensitive Response and Pathogenicity Genes

Research using *P. syringae pv. phaseolicola* has shaped our understanding of the T3SS. *hrp* mutants were first identified in the bean pathogen using Tn5 insertions (Lindgren *et al.*, 1986; Niepold *et al.*, 1985). The mutants lost pathogenicity to bean as well as the ability to elicit HR in tobacco. *hrp* genes have subsequently been shown to be conserved in other *P. syringae* pathogens, and are clustered on a pathogenicity island (PAI) that comprises at least six operons (Alfano *et al.*, 2000).

The transcripational regulation of components of the T3SS and effectors in *P. syringae pv. phaseolicola* has been studied in some detail (Thwaites *et al.*, 2004). After induction in minimal medium, quantitative real-time polymerase chain reaction (PCR) was used to analyse the transcription of three effector genes, *avrPphE*, *avrPphF* and *virPphA*, together with several *hrp* genes, including the first gene of each *hrp* operon and the regulators *hrpRS* and *hrpL*. A number of interesting observations were made, including that *hrpL* was rapidly induced, confirming its role as the key regulator of *hrp* gene expression. Further analysis comparing in *vitro* and in *planta* regulation of the same genes showed that transcript accumulation within the first 2 h after inoculation was considerably higher in the plant than in *vitro*, indicating that plant cell wall contact may enhance the transcription of T3SS and effector genes regulated by HrpL. Recently, Ortiz-Martin *et al.* (2010a, b) have described the positive and negative regulation of the Hrp region in strain 1448A. They analysed the roles of HrpL, HrpA and GacA in the positive regulation of T3SS, and Lon and HrpV in its negative regulation. Importantly, they showed that HrpL acts as a general regulator of gene expression, as it not only up-regulates non-T3SS-related virulence determinants, but also down-regulates flagellar function.

To identify additional genes involved in the regulation of the T3SS and effector genes, Deng *et al.* (2009) screened for mutants with reduced induction of luciferase-based reporter genes constructed with promoters of *hrpl* and the effector gene *avrPto*. Forty-six mutants were selected after screening approximately 28 000 transposon insertions. Most of the genes disrupted were

### Table 1 Race differentiation in *Pseudomonas syringae pv. phaseolicola*.

<table>
<thead>
<tr>
<th>Resistance alleles (R1–R5)†</th>
<th>Different cultivars</th>
<th>Races/avrulence genes (A1–A5)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5 6 7 8 9</td>
</tr>
<tr>
<td>Canadian Wonder</td>
<td>. . . .</td>
<td>1 . 1 . 1 .</td>
</tr>
<tr>
<td>S2 (ZAA54)</td>
<td>. . . .</td>
<td>. . . . . . . .</td>
</tr>
<tr>
<td>Tendergreen</td>
<td>. . 3 .</td>
<td>. 2 2 . . . . .</td>
</tr>
<tr>
<td>Red Mexican U13</td>
<td>1 . . 4 .</td>
<td>. . . . . . . .</td>
</tr>
<tr>
<td>1072</td>
<td>. 2 . . .</td>
<td>. . . . . . . .</td>
</tr>
<tr>
<td>A53 (ZAA55)</td>
<td>. . 3 . 4</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>A43 (ZAA12)</td>
<td>. 2 3 4 5</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>Guatemala 196-B</td>
<td>1 . 3 4 .</td>
<td>+ + + + + + + + + + + +</td>
</tr>
</tbody>
</table>

Adapted from Taylor *et al.* (1996a, b).

*The avirulence genes A1, A2 and A3 have been cloned as *avrPphF*, *avrPphE* and *avrPphB*, respectively.

†Current nomenclature used for the *R* genes is *Pse*-1 to *Pse*-4, and *pse*-5 (because this was found to be recessive), as described by Miklas *et al.* (2009).
found to encode metabolic enzymes, but three encoded regulatory functions. Further work revealed that the novel regulatory gene aefRwp3221 controlled both quorum-sensing and T3SS genes, leading to the suggestion that *P. syringae* pv. *phaseolicola* may regulate T3SS gene expression in response to cell density.

Harpins are a group of secreted effector proteins that travel through the T3SS in plant-pathogenic bacteria (Li et al., 2002). The *P. syringae* harpin, HrpZ1, encoded by *hrpZ* located within the *hrp* cluster, may operate as a 'helper' protein by facilitating the movement of effectors through the Hrp pilus. HrpZ1 also forms ion-conducting pores in membranes, and therefore may play a role in the delivery of effector proteins during infection (Lee et al., 2001). HrpZ1 is also recognized by plant cells as a pathogen-associated molecular pattern (PAMP) triggering immune responses (Chisholm et al., 2006). Recent work by Engelhardt et al. (2009) using HrpZ1 from *P. syringae* pv. *phaseolicola* has dissected the two properties of the protein and shown that ion pore formation and immune activation have different structural requirements. They showed that a C-terminal peptide fragment retains the ability to activate plant immunity, whereas ion pore formation requires intact HrpZ1.

Further analysis of the *hrp* cluster has focused on *hrcN*, encoding an ATPase that is related to the F$_1$-ATPase β subunit and catalyses protein translocation through the T3SS (Müller et al., 2006; Pozidis et al., 2003). The action of the HrcN ATPase is essential for export from the T3SS. The ATPase binds to and forms clusters in the bacterial inner member and can be extracted as a dodecamer (Pozidis et al., 2003). Müller et al. (2006) used cryo-electron microscopy to a resolution of 1.6 nm to reveal the three-dimensional structure of the dodecameric form of the T3SS ATPase of *P. syringae* pv. *phaseolicola*, and also proposed a model for the incorporation of the ATPase at the base of the secretion apparatus.

On the basis of their ability to utilize the T3SS pathway, effectors have been designated Hop, defining Hrp outer proteins (Alfano and Collmer, 1997). Lindeberg et al. (2005) proposed nomenclatural guidelines for type III Hop effector proteins in *P. syringae* to overcome the lack of an established systematic naming procedure. For example, AvrPphB, AvrPphE and AvrPphF are now also known as HopAR1, HopX and HopF1, respectively.

**ROLE OF EFFECTORS IN PATHOGENICITY**

Initial work on the determinants of pathogenicity in *P. syringae* used techniques such as transposon mutagenesis and heterologous library screening. The transposon insertion mutants isolated typically proved to be knockouts of *hrp* genes. Library screening, by the exchange of genomes between races, identified a number of *avr* genes, but no positive determinants of virulence (Mansfield, 2009). In 1999, a study was published that concentrated on the removal of plasmids from the bacterium and the examination of subsequent effects on pathogenicity (Jackson et al., 1999). A 154-kb plasmid (pAV511) was cured from *P. syringae* pv. *phaseolicola* strain 1449B by incompatibility with a cloned plasmid origin of replication. The resulting strain, RW60, lost virulence towards bean, causing the HR in previously susceptible cultivars.

Cosmid clones spanning a 30-kb region were found to restore virulence by complementation. This region contained a number of previously identified *avr* genes, including *avrD*, *avrB2* and *avrPphF*. Further sequence analysis revealed four potential virulence (*vir*) genes that were predicted to encode hydrophilic proteins and shared the *hrp*-box promoter motif indicating regulation by HrpL and, therefore, T3SS delivery. One gene achieved partial restoration of virulence when cloned on its own and was therefore designated *virPphA* (hopAR1). Interestingly, in soybean, *virPphA* acts as an *avr* gene controlling the expression of a rapid, cultivar-specific HR. The proximity of several *avr* and *vir* genes in the region that restored virulence led the authors to conclude that they had identified a plasmid-borne PAI. An additional interesting aspect of this work was that the loss of the plasmid led to the uncovering of a previously suppressed HR. This suggested that gene(s) encoding proteins able to trigger the HR are present elsewhere in the *P. syringae* pv. *phaseolicola* chromosome, but the functions of the plasmid-borne virulence genes were epistatic to the avirulence phenotype.

Subsequently, homologues of *virPphA* have been identified, namely *virPphA*$_{Pop}$ and *virPphA*$_{Ps}$ from *P. savastanoi* pathovars *glycinea* and *savastanoi*, respectively, and *avrPtoB* from *P. syringae* pv. *tomato* (Jackson et al., 2002). Sequencing showed that the *virPphA*$_{Po}$ homologue had a 48-bp central deletion in the open reading frame (ORF) compared with *virPphA* and *virPphA*$_{Ps}$, but otherwise all three of these alleles had >98% identity at the DNA level. By contrast, *AvrPtoB* from *P. syringae* pv. *tomato* was predicted to have only 51% amino acid similarity with *VirPphA*. However, *AvrPtoB* and *VirPphA* have been shown to share a C-terminal E3 ubiquitin ligase domain (Abramovitch et al., 2006). Each of the *virPphA* homologues was introduced into *P. syringae* pv. *phaseolicola* strain RW60, and all were shown to restore virulence to bean.

A second gene found on plasmid pAV511, *avrPphF*, was also shown to be capable of restoring virulence to *P. syringae* pv. *phaseolicola* strain RW60 in bean cv. Tendergreen. However, if this strain (RW60 containing *avrPphF*) was inoculated onto the universally susceptible bean cv. Canadian Wonder, HR was observed (Tsiamis et al., 2000). Moreover, another gene from pAV511, the soybean avirulence gene *avrPphC*, was found to block the activity of *avrPphF* in Canadian Wonder, but not in bean cv. Red Mexican. This work first revealed the complex web of interactions between *avr* and *vir* functions, highlighting the now commonly recorded suppression of the HR by effector proteins.
**EFFECOR EVOLUTION AND MOBILITY**

The effector gene *avrPphB* (*hopAR1*) was cloned by Jenner et al. (1991). *avrPphB* is chromosomally located and comprises an 801-bp ORF that is predicted to encode a cytoplasmic protein. The gene has a limited distribution, being present in races 3 and 4 of *P. syringae* pv. *phaseolicola*, but not in the numerous other *P. syringae* pathovars examined. The extensive study of *avrPphB* has provided interesting insights into effector evolution and function.

A variant of race 3 strain 1302A, named RJ3, exhibited an extended host range in cultivars of bean, and was found to have lost from the chromosome a large, 106-kb, region that contained *avrPphB* (Jackson et al., 2000; Pitman et al., 2005). The excised region shared similarity with genomic islands (GIs) found in other bacterial pathogens of animals as well as plants (Hacker and Carniel, 2001; Hacker and Kaper, 2000; van der Meer and Sentchilo, 2003), and was designated PPHGI-1.

Pitman et al. (2005) examined the dynamics of PPHGI-1 loss during infection and showed that, with repeatedpassaging of 1302A through resistant cv. Tendersgreen, the GI was quickly lost from the bacterial population. For example, after just three 1-week passages, 75% of bacterial colonies recovered from the plants caused disease symptoms, i.e. had lost *avrPphB*, whereas the remainder, as seen in the original 1302A, activated a rapid HR. This work showed how rapidly bacteria can evolve to overcome host resistance when interacting with the stressful environment of a resistant host plant (Arnold et al., 2007). PPHGI-1 is transferred between strains of *P. syringae* pv. *phaseolicola* (Lovell et al., 2009). Co-inoculation of donor 1302A and recipient 1448A strains into bean leaves led to the acquisition of an intact copy of PPHGI-1 in the chromosome of 1448A. It was also shown that the mechanism behind the transfer was transformation, because heat-treated cells or DNA extracts could also be used as donors. This process did not take place in vitro unless media were supplemented with apoplastic fluid extracted from bean leaves.

PPHGI-1 becomes a circular episome when it excises from the *P. syringae* pv. *phaseolicola* chromosome (Pitman et al., 2005) and, more recently, the circular form was found to be capable of independent replication (Lovell et al., 2011). When it is in the circular form, the expression of *avrPphB* is greatly reduced, leading to speculation that this may be a mechanism to prevent recognition of the Avr protein by the plant, and therefore allow time for the island to be reintegrated into the chromosome or transferred to another cell (Godfrey et al., 2011).

Deletion of *avrPphF* (*hopF1*) has also been implicated in the increased virulence of strains to cultivars carrying the matching *R1* resistance allele (Table 1). *avrPphF* is located on the 154-kb plasmid, pAVS11, in *P. syringae* pv. *phaseolicola* strain 1449B (Jackson et al., 1999). The PAI is generally well conserved in strains of *P. syringae* pv. *phaseolicola*, but *avrPphF* is sometimes lost as a result of large 9.5-kb or 10.5-kb deletions. The deletions are probably mediated by a chimeric mobile element composed of two novel insertion sequences, designated ISPsy16 and ISPsy17, that define the left border of each deletion (Rivas et al., 2005).

Small-scale changes have also been shown to occur in an effector sequence that lead to the avoidance of the plant’s surveillance machinery. The *P. syringae* pv. *phaseolicola* effector gene *avrPphE* (*hopX1*) is a good example. *avrPphE* was initially cloned from *P. syringae* pv. *phaseolicola* race 4 strain 1302A (Mansfield et al., 1994). Homologues of *avrPphE* are present in all nine races of *P. syringae* pv. *phaseolicola*, but *avr* phenotypes are only observed in races 2, 4, 5 and 7 (Stevens et al., 1998). Comparison of the sequence of the *avrPphE* alleles from the nine races of *P. syringae* pv. *phaseolicola* revealed single base-pair substitutions in races 1, 3, 6 and 9, which lead to single amino acid changes in the predicted translation product and abolish *avr* activity. The *avrPphE* allele from race 8 has a 104-bp insertion with a 12-bp inverted repeat at its borders. This insertion is predicted to produce a protein that is 60 amino acids longer than the wild-type and fails to induce the HR. Questions remain concerning the function of the AvrPphE variants that do not elicit the HR. Why are they maintained in the genome? Do they have a function in pathogenicity, or in another aspect of the *P. syringae* pv. *phaseolicola* life cycle?

**FUNCTION OF EFFECTORS**

Perhaps the most topical area of research into microbial pathogenicity concerns the biochemical functions of effector proteins. These studies have highlighted the clear similarities between mechanisms of pathogenicity in bacterial pathogens of both plants and animals. Whereas the primary amino acid sequence for most effector genes provides little insight into their biochemical function, structural analysis by crystallography has proved to be more rewarding.

The autoprocessed form of AvrPphB was the first reported crystal structure of a type III effector protein from a plant pathogen (Zhu et al., 2004). The protein is composed of a central antiparallel β-sheet, with α-helices packing on both sides to form a two-lobe structure. The core resembles papain-like cysteine proteases. AvrPphB and the *Yersinia pestis* effector YopT are now considered to define a family of 19 proteases found in pathogens of plants and animals (Shao et al., 2002). Their proteolytic activities are dependent on the invariant amino acids C139, H258 and D274. AvrPphB was shown to undergo autoproteolytic cleavage that required the C/H/D residues. This cleavage converted the wild-type protein from a 35-kDa into a 28-kDa form. These results were in line with earlier work of Puri et al. (1997), who showed that AvrPphB is processed in both
**Table 2** General features of the *P. syringae* pv. *phaseolicola* 1448A genome.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Chromosome</th>
<th>Plasmid p1448A-A</th>
<th>Plasmid p1448A-B</th>
<th>Characterization of selected ORFs</th>
<th>Number of ORFs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (bp)</td>
<td>5 928 787</td>
<td>131 950</td>
<td>51 711</td>
<td>Transport and binding proteins</td>
<td>728 (14)</td>
</tr>
<tr>
<td>G + C (%)</td>
<td>58.0</td>
<td>54.1</td>
<td>56</td>
<td>Cellular processes</td>
<td>708 (13)</td>
</tr>
<tr>
<td>Coding regions (%)</td>
<td>87.0</td>
<td>78.0</td>
<td>84.9</td>
<td>Regulatory role</td>
<td>535 (10)</td>
</tr>
<tr>
<td>No. of ORFs</td>
<td>5144</td>
<td>149</td>
<td>60</td>
<td>Mobile genetic elements</td>
<td>256 (5)</td>
</tr>
<tr>
<td>Mean ORF length (bp)</td>
<td>1007</td>
<td>660</td>
<td>719</td>
<td>Hypothetical proteins</td>
<td>224 (4)</td>
</tr>
<tr>
<td>No. of ORFs with annotated function (%)</td>
<td>3492 (68)</td>
<td>97 (62)</td>
<td>37 (62)</td>
<td>Conserved hypothetical proteins</td>
<td>822 (15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proteins of unknown function</td>
<td>681 (13)</td>
</tr>
</tbody>
</table>

ORF, open reading frame.
Adapted from Joardar et al. (2005).
ery of the proteins into plants containing the cognate R protein RPS2 (Guttman et al., 2002). Bioinformatic approaches have also been used to scan the genome sequences for features common to effector genes, e.g. screening for the consensus hrp-box motif, 5′-GGAACCNA-N13-14CCACNNNA-3′, which is a promoter sequence found upstream of effectors that are known to be regulated by hrpL, and also T3SS secretion signals. Using these parameters, Vencato et al. (2006) identified 27 high-probability candidates in P. syringae pv. phaseolicola 1448A.

To identify the complete complement of P. syringae pv. phaseolicola 1448A effectors, Zumaquero et al. (2010) analysed the translocation and/or expression of the 22 experimentally validated effectors plus other candidates to bring the confirmed effector inventory to 27. They also produced single- and double-knockout mutants of the majority of effectors, and used competitive index (CI) assays, comparing the multiplication of different strains within the same inoculated leaf, to investigate the individual contribution of effectors to virulence. For example, AvrB4-1 and AvrB4-2, which differ by three amino acids, were shown to be fully redundant and made a quantitative contribution to Pph1448A virulence in bean. The highly discriminatory CI assay may allow the contribution of individual effector genes to be revealed despite a background of functional redundancy.

**PHASEOLOTOXIN**

The name halo blight comes from the characteristic chlorotic halo that surrounds water-soaked lesions on a plant infected with P. syringae pv. phaseolicola. The chlorotic halo results from the action of phaseolotoxin, which is a nonspecific phytoxin. Phaseolotoxin inhibits ornithine carbamoyl transferase, a critical enzyme in the urea cycle (Moore et al., 1984). The production of phaseolotoxin is dependent on temperature, with 18–20 °C being optimal and no detectable amounts of the toxin being present at 30 °C (Nüske and Frischke, 1989). Transcription profiling has shown that the optimum expression of phaseolotoxin not only requires a low temperature, but also specific plant components present in leaf and apoplast (Hernández-Morales et al., 2009). The genes involved in phaseolotoxin biosynthesis are organized into the argK-tox gene cluster, which includes five transcriptional units (Aguilera et al., 2007; Peet et al., 1986). argK encodes a phaseolotoxin-resistant ornithyl carbamoyl transferase (Mosqueda et al., 1990). The argK-tox gene cluster comprises 23 genes, is flanked by insertion sequences and transposes and is located in a highly conserved 38-kb DNA fragment with the characteristics of a GI (Aguilera et al., 2007; Genka et al., 2006). The horizontal transfer of the cluster has never been demonstrated functionally, but its conservation in both P. syringae pv. phaseolicola and P. syringae pv. actinidiae indicates that it has been inherited by horizontal transfer (Sawada et al., 2002).

Significantly, the importance of phaseolotoxin in the bean pathogen was questioned in a study carried out in Castilla y León County, Spain (Rico et al., 2003). From a collection of 138 P. syringae pv. phaseolicola isolates taken from plants in commercial fields, 95 did not produce phaseolotoxin in vitro, as determined by an E. coli inhibition bioassay. Ninety-four of the isolates also failed to produce the expected PCR amplification product from primers designed to ORF6 of the phaseolotoxin gene cluster. This was the first report of the widespread occurrence of Tox⁻ isolates in the field, and has been confirmed by San José et al. (2010). Prior to this, it was considered that Tox⁻ strains were of little or no epidemiological significance.

**PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA AS A PROBE FOR DETERMINANTS OF NONHOST RESISTANCE**

Strains of P. syringae pv. phaseolicola have been used to probe mechanisms of nonhost resistance in Arabidopsis. Interestingly, despite encoding numerous effectors (Block et al., 2008; Cunnac et al., 2009; Joardar et al., 2005), P. syringae pv. phaseolicola typically does not cause the HR in Arabidopsis accessions, although ectopic expression of avr genes will, in some cases, activate the programmed cell death response. Microscopy of defence responses was carried out by Soylu et al. (2005), who examined the HR induced by P. syringae pv. phaseolicola strain 1448A harbouring the avr genes avrPpiA and avrPphB (matching R genes RPM1 and RPS5, respectively; Grant et al., 1995; Simovich and Innes, 1995), as well as basal, cell wall-associated defences. It has been argued that the response of Arabidopsis to PAMPs from P. syringae pv. phaseolicola, notably flagellin, may be so rapid that it blocks the activation of the HR, perhaps because of the prevention of delivery of effector proteins (Forsyth et al., 2010).

Lu et al. (2001) used P. syringae pv. phaseolicola strain NPS3121 to screen mutants of the A. thaliana Columbia (Col-0) accession for increased susceptibility to colonization. Their detailed study resulted in the cloning of the nonhost resistance 1 (NHO1) gene that was found to encode a glycerol kinase (Kang et al., 2003). Expression of NHO1 is increased rapidly after bacterial challenge, but the precise role of the glycerol kinase, as a component of the mechanism of defence, has not been resolved (He et al., 2006).

Using single and double mutants with disruptive insertions in known defence genes, Ham et al. (2007) examined their roles in resistance to P. syringae pv. phaseolicola strain NPS3121. Their results showed that mutations in individual genes, pmr4, pad4 and npr1, did not affect the ability of P. syringae pv. phaseolicola to multiply in Col-0 leaves, but the double mutant pad4/pmr4 allowed enhanced colonization, particularly if NPS3121 expressed the effector HopM1. They concluded that nonhost
resistance was a ‘multilayered’ process controlled by several genes regulating different signalling pathways and mechanisms of resistance.

Expression of the effector AvrPtoB in the RW60 strain, derived from *P. syringae pv. phaseolicola* strain 1449A (Jackson et al., 1999), also reduces resistance particularly in the *Arabidopsis* Wassilewskija (Ws) accession but not in Niedersenz (Nd; de Torres et al., 2006). In contrast to Nd or Col, Ws was found to be weakly susceptible to *P. syringae pv. phaseolicola* strain 1448A (Forsyth et al., 2010). The strong resistance of Nd was attributed to the presence of FLS2 encoding the flagellin receptor (which is absent from Ws) and a second gene that was mapped to chromosome 1. Flagellin from *P. syringae pv. phaseolicola* seems to be very effective in eliciting resistance in *Arabidopsis* and, although other potential sensors may contribute to defence activation (for example, the receptor-like protein ATRLP30; Wang et al., 2008), the receptor FLS2 represents the major barrier to infection.

DISEASE CONTROL AND CONCLUDING REMARKS

Taylor et al. (1979) highlighted the importance of using disease-free seed for the control of outbreaks of halo blight, pointing out that there are no bacteriocidal sprays of lasting value available for control once the disease has become established. Taylor et al. have also shown the importance of the selection of varieties with resistance to the prevalent pathotype of *P. syringae pv. phaseolicola* (Taylor et al., 1996a, b). The current knowledge of race structure has allowed some *avr* genes to be genotyped using PCR-based methods and provides a reliable indication of the likely success of certain cultivars. Unfortunately, although some genotypes appear to show reduced susceptibility, there are no varieties of bean highly resistant to the most prevalent race 6 (Terán et al., 2009).

Clean seed is essential and molecular probes have been developed to allow the detection of seed-borne inoculum. One approach has been to use PCR or serological protocols that detect the phaseolotoxin gene cluster or its products. However, reports of *Tox* strains in diseased crops have brought into question the practice of targeting phaseolotoxin as a biomarker (Rico et al., 2003; San José et al., 2010). An alternative method has recently been reported using a TaqMan probe and primer set based on a site-specific recombinase gene (Cho et al., 2009). Li et al. (2009) have developed a loop-mediated isothermal amplification (LAMP) protocol for rapid and specific characterization and identification of *P. syringae pv. phaseolicola* from other pathovars of *P. syringae* and other plant-associated bacteria. The additional methods should allow the rapid detection of all *P. syringae pv. phaseolicola* pathotypes. The use of molecular probes is therefore already having an impact on disease control strategies.

Where next for the ‘supermodel’? We suggest that the value of *P. syringae pv. phaseolicola* as a model Gram-negative pathogen will continue to be realized in the following five areas of basic understanding of plant–pathogen interactions.

1. The contribution of effectors to host specificity will become more apparent from genomic analyses coupled with functional assessment of the targets of individual effectors. Analysis of the variation in virulence of *P. syringae pv. phaseolicola* to diverse legumes should prove particularly informative.
2. The dissection of plant defences will be achieved using effectors from the halo blight bacterium that have defined roles in the suppression of *R* gene-based defences and also PAMP-triggered immunity.
3. The involvement of effectors in harnessing nutrients from the invaded plant should become more apparent, particularly through research on the prolonged biotrophic infections that are able to develop in pods (Harper et al., 1987).
4. The unexpected plasticity of genomes, as revealed from experiments on the dynamics of PPHGI-1 (Lovell et al., 2011), should be examined in depth using high-throughput sequencing platforms. It would be intriguing to understand the frequency of occurrence of genome rearrangements and the contribution they make to the evolution of new strains of the pathogen.
5. An understanding of the spatial dynamics of genome rearrangement within the infected plant will become possible using the fluorescent tagging strategies developed by Godfrey et al. (2011). Bacteria tagged with fluorescent proteins will also be invaluable in attempts to develop mathematical models of effector delivery and plant responses occurring during colonization.

These are exciting times for molecular plant pathology and there is little doubt that research using the halo blight pathogen will lead to further advances in our understanding of microbial pathogenicity and plant defences. It is to be hoped that the more basic research ongoing that uses *P. syringae pv. phaseolicola* as a model bacterial pathogen will have spin-offs in control, particularly in countries in which beans represent part of the staple diet.

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