Lifestyles of the Effector Rich: Genome-Enabled Characterization of Bacterial Plant Pathogens[1]

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Virtually all crop plants are attacked by pathogenic microbes, including bacteria, fungi, oomycetes, and nematodes. In many cases, bacterial diseases are still poorly controlled with century-old agents like copper, and cause serious losses, as seen with the recent citrus canker outbreak in Florida (Schubert et al., 2001). Pathogenicity has evolved independently in diverse phylogenetic lineages within the bacteria, resulting in a range of pathogenic lifestyles that parallels those that have evolved in the fungi and oomycetes. Thus, the study of bacterial pathogens is casting light into the interactions of plants with all microbes. This window of investigation was flung open in 2000 with publication of the first complete genome sequence of a phytopathogen, *Xylella fastidiosa* 9a5c (Simpson et al., 2000). Because it is a xylem-limited, nutritionally fastidious pathogen, its mechanisms of pathogenicity had been a complete mystery prior to analysis of the genome sequence. Subsequent sequencing of experimentally tractable pathogens in the genera *Ralstonia* (Salanoubat et al., 2002), *Xanthomonas* (Da Silva et al., 2002), *Pseudomonas* (Buell et al., 2003), and *Pectobacterium* (Bell et al., 2004) had a similarly revolutionary impact, revealing unexpected complexities in their virulence systems.

Of particular importance to plant biologists is the genome-enabled, comprehensive identification of proteins and toxins that directly interact with plants and are referred to here as effectors (Hogenhout et al., 2009). Because effectors act during infection and outside of the bacterium, genes encoding them can be systematically identified on the basis of expression in planta and passage of their products through secretion pathways known to be trafficked by virulence proteins. Of the seven secretion systems described to date in gram-negative bacteria, the type II secretion system (T2SS) and type III secretion system (T3SS) are responsible for extracellular localization of the majority of critical virulence factors. *Agrobacterium tumefaciens*, an important pathogen that relies on the type IV pathway to deliver nucleoprotein T-DNA complexes into plant cells, has also been sequenced (Goodner et al., 2001; Wood et al., 2001) and is addressed in another *Update* article in this *Focus* issue. Small molecule effector candidates, which do not typically rely on a dedicated secretion system, can be identified through characterization of nonribosomal peptide and polyketide synthetases identified in the genome (Bender et al., 1999). Since each effector interacts with at least one plant molecule, and these targets involve diverse organelles and metabolic processes, the newly expanded collection of effectors provides a vast new tool box for plant biologists, revealing the unexpected extent of pathogen manipulations of plants (Speth et al., 2007). Importantly, complete genome sequences also enable investigation of how all of the effector parts work together to enable parasitism in different plants and specific plant niches.

This *Update* highlights insights gained from fully sequenced bacterial pathogen genomes that are of particular relevance to plant biologists. We will describe the range of bacterial phytopathogens and their lifestyles in plants, lessons gained from type III effector repertoires (a focus of much study during this period), major insights arising from each of the phytopathogen groups with completely sequenced genomes, and future challenges.

THE DIVERSITY OF PHYTOPATHOGENIC BACTERIA AND AN EMERGING BIG PICTURE OF THEIR INTERACTIONS WITH PLANTS

Plant pathogens of all classes are now considered to have two broadly different pathogenic lifestyles, with necrotrophs gaining nutrients from rapidly killed tissue and biotrophs gaining nutrients from living host tissue (or in the case of hemibiotrophs, from living tissue that dies in a later stage of pathogenesis; Glazebrook, 2005). The phytopathogenic bacteria with complete and published sequences span this continuum of pathogenic lifestyles, summarized in Table I.

The soft-rot enterobacterium *Pectobacterium atrosepticum* SCRI1043 (Bell et al., 2004) was the first sequenced representative of the necrotrophs. The pioneer hemibiotrophs were *Ralstonia solanacearum*

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Table I. *Salient properties of representative phytopathogenic bacteria with published genome sequences*

Host names followed with an ellipsis denote a broader host range. Column headings: V, obligately vectored by insects (as denoted by +); T, tissue primarily colonized in plant (M, mesophyll; X, xylem; P, phloem); CW, cell wall-degrading enzymes; TE, translocated effectors acting within plant cells; SP, extracellular Ser proteases; SM, small molecules. Symbols in Effectors columns: +, one or a few present; ++ many present and major role; −, none present; blank cell, not studied. References are in text.

Bacterial Groups and Representative Pathogens	Disease	Host(s)	V	T	Effectors			
					CW	TE	SP	SM
Proteobacteria (necrotrophs)								
P. atrosepticum	Black leg	Potato		MX	++	+		+
Proteobacteria (hemibiotrophs)								
P. syringae pv *tomato*	Bacterial speck	Tomato, Arabidopsis, other Brassica		M	+	++		+
R. solanacearum	Bacterial wilt	Tomato, Arabidopsis…		X	++	++		+
X. axonopodis pv *citri*	Canker	Citrus		M	++	++		
X. campestris pv *campestris*	Black rot	Arabidopsis, other Brassica		X	++	++		
X. fastidiosa	Variegated chlorosis	Citrus	+	X	+	−		
Actinobacteria								
C. michiganensis subsp. *michiganensis*	Wilt and canker	Tomato		X	+	−	++	
C. michiganensis subsp. *sepedonicus*	Ring rot	Potato		X	+	−	++	
L. xyli subsp. *xyli*	Ratoon stunting	Sugarcane	+	X	+	−	+	
Firmicutes								
Candidatus *P. asteris*	Yellows	Wide host range	+	P	−	+		

GMI1000 (Salanoubat et al., 2002), *Xanthomonas campestris* pv *campestris* ATCC33913 (Da Silva et al., 2002), *Xanthomonas axonopodis* pv *citri* 306 (Da Silva et al., 2002), and *Pseudomonas syringae* pv *tomato* DC3000 (Buell et al., 2003). Although sharing the basic property of dependence on type III effectors, the hemibiotrophic pathogens have interesting differences in their interactions with plants. For example, *R. solanacearum* is a wilt pathogen with a potentially broad host range that colonizes the xylem after entering plants through roots. *X. campestris* pv *campestris* also can colonize the xylem, but it attacks aerial parts of the plant and has a narrow host range. In further contrast, *X. axonopodis* pv *citri* and *P. syringae* are narrow-host-range pathogens that colonize the mesophyll rather than the xylem. All of these bacteria are gram negative, with *R. solanacearum* in the β-Proteobacteria and the others in the γ-Proteobacteria.

Clavibacter michiganensis subsp. *michiganensis* NCPPB382 (Gartemann et al., 2008), *C. michiganensis* subsp. *sepedonicus* ATCC33113 (Bentley et al., 2008), and *Leifsonia xyli* subsp. *xyli* CTCB07 (Monteiro-Vitorello et al., 2004) are the pioneer, sequenced bacteria in the high G + C content, gram-positive group Actinobacteria. These bacteria are closely related and are all xylem colonizers. Analogous to *X. fastidiosa*, *L. xyli* subsp. *xyli* is a fastidious bacterium with a small genome size (2.6 Mb) that appears to be a product of reductive evolution (Moreira et al., 2004). The end point in the continuum toward biotrophic specialization for life within a specific plant niche and associated genome reduction is represented by wall-less bacteria in the low G + C content, gram-positive group, Firmicutes. These bacteria are obligate colonizers of the phloem and their phloem-feeding insect vectors (Hogenhout and Loria, 2008). Notably, the Candidatus *Phytoplasma asteris* OY-M pioneer genome is only 860 kb (Oshima et al., 2004). In contrast, the largest genome among this pioneering set of phytopathogens is that of *P. syringae* pv *tomato* DC3000, which is 6.5 Mb.

A conceptually and agriculturally significant feature of these pathogenic lifestyles and phylogenetic groups is that the plant defenses against necrotrophs and (hemi)biotrophs are different and antagonistic. Specifically, major gene resistance is only effective against hemibiotrophic gram-negative bacteria injecting type III effectors that are recognized by cytoplasmic resistance (R) proteins in the host (Spoel et al., 2007; Poland et al., 2009). A recently formulated model for the interactions of plants with hemibiotrophic Proteobacteria has broad explanatory power (Jones and Dangl, 2006; Gohre and Robatzek, 2008). According to this model, bacteria in the apoplast display pathogen (or microbe)-associated molecular patterns (PAMPs), such as flagellin, lipopolysaccharide, peptidoglycan, and elongation factor EF-Tu, which are recognized at the plant cell surface by pattern recognition receptors that elicit PAMP-triggered immunity (PTI). Pathogens defeat this defense by injecting type III effectors that suppress PTI. The T3SS, required for delivery of these effectors and thus essential to virulence in all hemibiotrophic Proteobacteria pathogens, is discussed in another *Update* in this issue. Although effectors suppress PTI, they may also elicit defenses. Specifically, if the host carries an appropriate *R* gene for detecting the activity of one or more of these effectors inside plants cells, effector-triggered immunity (ETI) is activated, typically manifested as a defense-related programmed cell death referred to as the hypersensitive response. PTI is addressed in more detail in another *Update* in this issue but this model, based on plant interaction with hemibiotrophic Proteobacteria, raises important questions about other groups of phytopathogenic bacteria. Are PAMPs also perceived in the xylem or the

phloem? How does *Clavibacter*, which does not appear to inject effectors, evade or suppress PTI? How do necrotrophs defeat PTI? What determines the differing levels of host specificity and tissue specificity observed in various pathogen groups?

THE TYPE III EFFECTOR REPERTOIRES OF HEMIBIOTROPHIC PROTEOBACTERIA

The greatest impact of bacterial phytopathogen genome sequencing so far has been the discovery of large numbers of type III effectors in various phytopathogenic Proteobacteria. Because of gene duplication and functional redundancy, many type III effectors are individually dispensable. In hindsight, it is not surprising that the corresponding genes were largely missed in pregenomics era screens for mutants with reduced virulence. Genomics bypassed this problem by enabling various functional screens and characterization of sequence patterns to identify all probable candidates in each strain, many of which have been experimentally validated. Patterns used have included promoter motifs, amino acid biases associated with type III targeting signals, motifs predicting eucaryote-like functions, and presence in genomic islands (Cunnac et al., 2004; Lindeberg et al., 2006; Vinatzer and Yan, 2008; Furutani et al., 2009). These efforts have revealed complex effector repertoires for several strains, as well as provisional super repertoires for the pangenomes of three phytopathogen groups, as tabulated in recent reviews addressing *Xanthomonas* spp. (Kay and Bonas, 2009), *R. solanacearum* (Poueymiro and Genin, 2009), and the *P. syringae* pathovars (Cunnac et al., 2009).

Effector repertoires are highly variable, even for pathogens of a single plant species. The relative size and variability of these repertoires can be seen in three well-studied tomato (*Solanum lycopersicum*) pathogens: *X. campestris* pv *vesicatoria* 85 to 10 (17 effectors confirmed), *R. solanacearum* GMI1000 (28 confirmed + 46 candidates), and *P. syringae* pv *tomato* DC3000 (28 confirmed; Cunnac et al., 2009; Kay and Bonas, 2009; Poueymiro and Genin, 2009). Of the 17 effectors injected by *X. campestris* pv *vesicatoria*, five have homologs in *R. solanacearum* and three have homologs in *P. syringae* pv *tomato* (Kay and Bonas, 2009). This variability in type III effector repertoires is particularly striking in two strains of *P. syringae* pv *tomato*: DC3000 and T1. Both strains cause bacterial speck of tomato, but only half of their effector repertoires are shared (Almeida et al., 2009). Complete genome sequences are now published for seven *Xanthomonas* strains representing diverse species and pathovars, and three pathovars of *P. syringae* (Da Silva et al., 2002; Buell et al., 2003; Feil et al., 2005; Joardar et al., 2005; Lee et al., 2005; Qian et al., 2005; Thieme et al., 2005; Salzberg et al., 2008; Vorholter et al., 2008). Comparison of their effector repertoires similarly reveals a high degree of variability, with no obvious correlation between repertoire composition and host specificity or tissue specificity (Salzberg et al., 2008; Almeida et al., 2009). Thus, it appears that there are many ways for bacteria to defeat plant defenses with type III effectors.

Enormous progress has been made in identifying the diverse biochemical activities, subcellular targets, and host interactors of type III effectors. These properties of individual effectors are summarized in recent reviews (Block et al., 2008; Gohre and Robatzek, 2008; Cunnac et al., 2009; Kay and Bonas, 2009; Poueymiro and Genin, 2009), and we are limited here to a few highlights that indicate the scope and sophistication of the effector assault on plants. In general, type III effectors manipulate host cell protein turnover, RNA synthesis and stability, and protein phosphorylation (Block et al., 2008). Effectors can insert themselves into host protein processing and targeting pathways, resulting in localization to the plasma membrane, chloroplasts, the nucleus, and other subcellular sites (Gohre and Robatzek, 2008; Dowen et al., 2009). Their molecular targets can range from pattern recognition receptors essential for PAMP perception to promoters for genes controlling cell size (Gohre and Robatzek, 2008). The biological function of most type III effectors appears to be suppression of PTI and/or ETI, and some effectors, such as AvrPtoB, have multiple domains that suppress both defenses (Abramovitch and Martin, 2005; Rosebrock et al., 2007). The bewildering range of activities and subcellular and molecular targets of type III effectors is best appreciated by scanning the tables of recent reviews (as cited above). However, it is worth noting that only a small fraction of the known effectors have been characterized, and the known effectors represent only a fraction of the total that are likely encoded in the pangenomes of the hemibiotrophic Proteobacteria.

THE IMPORTANCE OF REDUNDANT EFFECTOR GROUPS IN PHYTOPATHOGENIC BACTERIA

The model described above of pathogenesis based on translocated effectors suppressing PTI while being under R protein surveillance evokes a coevolutionary war between plants and pathogens that can generate large and polymorphic repertoires of effectors and R proteins (McHale et al., 2006; Stavrinides et al., 2008). Importantly, this process appears to generate effector repertoires that are collectively essential but where individual effectors are typically dispensable. Because of this dispensability, effector genes can be lost with little or no virulence penalty by pathogen populations facing a cultivar that relies on *R*-gene-mediated resistance. Thus, *R*-gene-mediated resistance is often defeated after a few years of agricultural use, as has been observed with many important bacterial, fungal, and oomycete pathogens (Jones and Dangl, 2006; Poland et al., 2009).

The availability of complete genome sequences and complete type III effector repertoires has enabled investigation of an important property of most effector

repertoires, that is, the dispensability of individual effectors. Such dispensability has been inferred from the results of numerous mutant screens involving various hemibiotrophic Proteobacteria and has been systematically explored with *R. solanacearum* GMI1000 and *P. syringae* pv *tomato* DC3000. Mutagenesis of 42 effector and effector candidate genes in GMI1000 revealed that only two had a virulence phenotype in host tomato, manifested as only a slight delay in symptom development (Cunnac et al., 2004). Similarly, a screen of *P. syringae* pv *tomato* DC3000 transposon mutants with a sensitive virulence assay based on dip-inoculated Arabidopsis (*Arabidopsis thaliana*) plants yielded only a single effector gene, which only quantitatively contributes to lesion formation (Brooks et al., 2004; Badel et al., 2006).

Through the efforts of multiple research groups, the type III effector repertoire of DC3000 has been particularly well established and is thought to comprise 28 actively deployed effectors (Lindeberg et al., 2006). Combinatorial deletions involving 20 of the active effector genes have revealed a redundancy-based structure in the effector repertoire, such that some deletions diminish growth in planta only in combination with other deletions (Kvitko et al., 2009). It was found that two redundant effector groups are particularly important in promoting DC3000 growth in planta, and based on the known activities of some of the members, these internally redundant groups were proposed to target different high-level processes in PTI: perception of PAMPs and vesicle trafficking of antimicrobial factors. These observations suggest that successful pathogenesis by DC3000 depends on blocking of a few key defense processes, with each process targeted redundantly. Host surveillance of the effectors and, possibly, the polymorphic nature of effector targets may explain the apparent need for redundancy.

The phenomenon of redundant effector groups appears widespread in phytopathogenic bacteria. For example, the extracellular components of the T3SS that have been genetically implicated in forming the translocation pore in the host plasma membrane are conserved and individually essential in animal pathogens but more numerous, variable, and individually dispensable in plant pathogens (Kvitko et al., 2007). *R. solanacearum* and many *Xanthomonas* spp. also have candidate redundant effector groups represented by the multiple copies of F-box-containing GALA family effectors and transcription activator-like effectors that their respective genomes encode (Angot et al., 2006). Experiments thus far with both effector families indicate that combinatorial mutations are required to produce significant virulence phenotypes (Yang et al., 1996; Angot et al., 2006). As will be discussed in the next section, redundant groups of candidate effectors (broadly defined) can also be found in *Pectobacterium* and *Clavibacter* spp.

Systematic identification of redundant effector groups has the potential to reveal processes within plants that contribute to defense against microbial pathogens and may provide clues to the specific plant proteins targeted by and/or involved in the recognition of individual effectors. For example, AvrPto and AvrPtoB, though showing no sequence similarity, are members of a redundant effector group that inhibits the kinase activity of the FLS2 pattern recognition receptor and interacts with Pto, now thought to function as a decoy kinase under R protein surveillance (Shan et al., 2008; Xiang et al., 2008; Zhou and Chai, 2008). Identification of redundant effector groups may also have practical utility in suggesting more durable combinations of *R* genes less likely to be overcome by pathogen mutation. Ultimately, the genomics-enabled characterization of type III effector repertoires and interacting plant proteins is serving to unravel the complex network of host-pathogen interactions in a manner that is biologically significant and agriculturally useful.

OTHER LESSONS FROM PATHOGEN GENOMES

The *P. atrosepticum* genome highlights two other important classes of effectors: pectic enzymes and phytotoxins. Like other soft-rot enterobacteria, *P. atrosepticum* (formerly *Erwinia carotovora* subsp. *atroseptica*) produces multiple pectic enzymes, secreted via the T2SS (Toth and Birch, 2005). Studies with various soft-rot enterobacteria involving mutations in T2SS genes and either single or multiple pectic enzyme genes suggest that pectic enzymes are collectively essential but individually dispensable for the maceration of parenchymatous plant tissues characteristic of soft-rot diseases (Toth et al., 2006). The *P. atrosepticum* genome sequence revealed 11 new putative pectic enzyme genes, thus bringing the repertoire to 20 (Bell et al., 2004). However, there is no evidence that plant surveillance or inhibitors are driving amplification of the *P. atrosepticum* pectic enzymes or causing diversification in the repertoire encoded by related species (Glasner et al., 2008). Subtle specialization for substrates and reaction conditions in the cell walls of different plants may account for the amplification of effectors in this class. In contrast, T2SS mutants are only partially reduced in virulence, and pectic enzymes appear to have only a minor role in *P. syringae* pathogenesis (Bauer and Collmer, 1997; Bronstein et al., 2005).

A surprising discovery in the *P. atrosepticum* genome was the presence of homologs of *P. syringae* genes directing biosynthesis of the toxins syringomycin and coronafacic acid (Bell et al., 2004), mutations that strongly reduced black leg disease in potato (*Solanum tuberosum*; Bell et al., 2004). In *P. syringae* pv *tomato* DC3000, coronatine, an amide-linked conjugate of coronafacic acid and an Ile derivative, mimics jasmonic acid Ile, promotes the opening of stomates and bacteria entry, and suppresses salicylic acid-dependent plant defenses (Brooks et al., 2005; Melotto et al., 2008). The biosynthetic capacity for this toxin family is encoded in a horizontally acquired region in DC3000 and is present in only a subset of other *P. syringae* pathovars and in

only *P. atrosepticum* among the sequenced *Pectobacteria* spp. (Hwang et al., 2005; Glasner et al., 2008; Lindeberg et al., 2008). Like *P. syringae*, *P. atrosepticum* encodes a functional T3SS, but the only effector candidate is a homolog of the widespread *P. syringae* effector AvrE (Bell et al., 2004). In summary, although *P. atrosepticum* and *P. syringae* are in different bacterial families, they appear to have acquired effector genes by horizontal transfer, with their respective effector repertoires being differently expanded in association with their distinct pathogenic lifestyles.

Comparison of phylogenetically divergent organisms with similar pathogen lifestyles provides yet another opportunity for exploring the nature and evolution of bacterial pathogenesis through comparative genomics. For example, the >50 *P. syringae* pathovars and >100 *Xanthomonas* species/pathovars are all T3SS-dependent, host-specific pathogens that are often good epiphytes and commonly cause diseases characterized by scattered lesions on foliage (although some *Xanthomonas* spp. also invade the xylem and cause extensive tissue death). Most crops are attacked by at least one member of each group. Importantly, genome sequencing suggests that phytopathogenicity in these two genera has evolved convergently. For example, not only are their type III effector repertoires largely different, but they also possess independently acquired and distinct T3SS (Alfano and Collmer, 1997). Thus, functional genomic comparisons of strains with common hosts from the parallel pseudomonad and xanthomonad series could indicate the potential range of interactions plants may have with microbes that attack via translocated effector proteins.

R. solanacearum represents a versatile pathogen, unique among the major bacterial phytopathogens given its ability to attack plants via the roots (Genin and Boucher, 2004) and particularly devastating to a variety of tropical crops. The bacterium has large repertoires of candidate effectors traveling the T2SS and the T3SS, and appropriate mutants indicate that the two pathways and their respective repertoires are important for pathogenesis (Poueymiro and Genin, 2009). The type III effector repertoire features three expanded families with multiple members as well as multiple effectors with repeat domains implicated in the recognition of host proteins (Poueymiro and Genin, 2009). The GALA proteins mentioned above provide a good example, with strain GMI1000 producing seven such proteins predicted to target host proteins for degradation via the 26S proteosome (Angot et al., 2006). Analogous to *P. syringae* pv *tomato* in its manipulation of hormone signaling with a small-molecule effector, GMI1000 produces ethylene, which affects the expression of ethylene-response host genes during infection (Valls et al., 2006).

Genomic analyses suggest that effector families may be similarly subject to amplification in gram-positive pathogens as well. Pregenomics research had identified two *C. michiganensis* subsp. *michiganensis* proteins, CelA endo-β1 to 4 glucanase and Pat-1 Ser protease (Hogenhout and Loria, 2008), which appear to be effectors given their role in virulence and predicted extracellular location. One or more yet to be identified proteins in the extracellular fluids from both subspecies have additionally been shown to elicit the hypersensitive response in tobacco (*Nicotiana tabacum*; Nissinen et al., 1997; Alarcon et al., 1998). Genome sequences reveal that homologs of *celA* family members and *pat-1* family members are present in subspecies *michiganensis* and *sepedonicus*, as well as in *L. xyli* subsp. *xyli* (Monteiro-Vitorello et al., 2004; Bentley et al., 2008; Gartemann et al., 2008). Intriguingly, subspecies *michiganensis* encodes at least 28 Ser proteases of which 10 are Pat-1-like members of the Chp family. These gene families have several characteristic properties of effector genes including an atypical G + C content suggestive of horizontal acquisition and a demonstrated role in elicitation of the hypersensitive response on nonhost plants for at least one of the family members. Subspecies *sepedonicus* harbors 11 Chp family genes, and *L. xyli* subsp. *xyli* has one. It is tempting to speculate that the Chp family Ser proteases are acting extracellularly to degrade either pattern recognition receptors or antimicrobial peptides/proteins and that ETI surveillance of these proteins or their activities has driven amplification of the family. The Chp Ser proteases thus could provide new clues to how plants defend the xylem against pathogens. It is also noteworthy that *C. michiganensis* subsp. *michiganensis* and *L. xyli* subsp. *xyli* encode tomatinase and a fatty acid desaturase, respectively. Tomatinase can release defense-suppressive products from the antifungal saponin tomatine (Bouarab et al., 2002), and the fatty acid desaturase may produce abscisic acid, which could contribute to the major symptom of ratoon stunting of sugarcane (*Saccharum officinarum*; Monteiro-Vitorello et al., 2004). Thus, both of these proteins have the potential to generate small-molecule effectors.

In contrast to the other pathogens discussed, the phytoplasmas can inhabit the intracellular space of both insects and plants and may evade plant defenses in large part through absence of PTI-triggering PAMPs that have been lost during genome reduction. Nonetheless, identification of extracellular effector candidates on the basis of conserved targeting motifs (in this case, N-terminal signal peptides facilitating secretion via a functional Sec pathway) represents a valuable approach for effector identification. Eukaryotic nuclear localization signals have been identified in two of the effector candidates with SAP11 from Candidatus *P. asteris* AY-WB shown to induce necrosis and alter transcription (Bai et al., 2006; Hogenhout and Oshima, 2008).

NEW CHALLENGES

A primary challenge of genomics research involves discovery of patterns in the DNA and protein sequences. Fortunately, because typical effector genes encoding type III effectors, cell wall-degrading enzymes, extracellular proteases, and biosynthetic en-

zymes for toxins and phytohormones carry a variety of predictive patterns, we have been able to make substantial progress toward comprehensive identification of effectors in the pioneer pathogen genomes. The subsequent sequencing of strains related to the pioneers has enabled a second round of pattern searching focused on differences in virulence and host or tissue specificity and an initial glimpse of the pangenomes for several species. In the near future, we can expect publication of pioneer genome sequences for other important pathogens, such as *Streptomyces scabies*, *Erwinia amylovora*, *Pantoea stewartii*, and *Dickeya dadantii* (formerly *Erwinia chrysanthemi*). Furthermore, next-generation sequencing methods have the potential to yield low-cost draft genomes for a virtually unlimited set of relatives for each pioneer. These advances, coupled with continuing refinements in the iterative process of effector function analysis and pattern recognition, should yield the complete effecterome for each of these pathogen groups.

The next fundamental challenge will be to discern patterns in these effector repertoires that underlie their evolutionary assembly into viable systems for defeat of host defenses and adaption to plant-associated niches. For example, it appears that diverse phytopathogenic bacteria (with the possible exception of the Firmicutes) produce both protein effectors and small molecule effectors. Do these two classes of effectors work coordinately? And, how do effector repertoires function in coordination with the rest of the bacterial genome and physiology? In this regard, it is important to note that effector repertoire composition has so far failed to explain either the host or tissue specificity of different members of the hemibiotrophic Proteobacteria. Furthermore, although R protein surveillance of type III effectors certainly explains race-cultivar specificity in the field, it may not explain the specificity of *P. syringae* pathovars, *R. solanacearum* strains, or *Xanthomonas* spp. for their different plant species. The latter specificity is generally stable in the field despite the observation that loss of just one or two effectors can expand host range to new plant species (Castaneda et al., 2005; Lin and Martin, 2007; Wei et al., 2007; Poueymiro et al., 2009). It is possible that multiple adaptations involving PAMP perception, nutrition, and antimicrobial factors underlie host and tissue specificity.

The ultimate challenge of effector identification and functional characterization involves the integration of their various individual roles into a comprehensive picture of host-pathogen interaction. As one means of managing the increasingly complex data on DC3000 effectors, Gene Ontology annotation is being used to systematically document the biological processes, molecular functions, and cellular locations of individual effectors, enabling comparison among effectors deployed by a single strain as well as among those deployed during the course of other, diverse host-pathogen interactions.

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