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2.1 Introduction

2.1.1 Soft Rot *Enterobacteriaceae* Taxonomy and Phylogeny

The two genera in the soft rot *Enterobacteriaceae* (SRE), *Pectobacterium* and *Dickeya*, have had many names over the past 110 years, with each change reflecting growth and precision gained in knowledge about phytopathogens. Like many bacterial genera, the SRE were originally described as *Bacillus* (Jones 1900, 1901), but they were soon moved to the new genus, *Erwinia*, the name of which was derived from one of the founders of phytobacteriology, Erwin Frink Smith (Winslow et al. 1917). For many decades, the genus *Erwinia* was used for all plant pathogenic *Enterobacteriaceae* despite clear evidence that these species were not monophyletic. As early as 1945, taxonomists proposed to move *Erwinia carotovora* and other SRE into a new genus, *Pectobacterium*, to reflect differences in its physiology (Waldee 1945), but this new name

was not generally accepted until more extensive phylogenetic work was completed, almost 100 years after this group of plant pathogens was first described (Gardan et al. 2003; Hauben et al. 1998; Kwon et al. 1997) (Table 2.1). Soon afterward, another SRE species, *Erwinia chrysanthemi*, was placed into the new genus *Dickeya* and at the same time divided into multiple species (Brady et al. 2012; Samson et al. 2005).

The two genera that comprise the SRE may be phylogenetically separated by the genus *Brennaria*, which contains non-pectolytic strains that infect mainly trees (Brady et al. 2012). Although *Brennaria* genome sequences are available, the genomic differences among the *Pectobacterium*, *Dickeya*, and *Brennaria* remain little explored. Little is known about the biology of *Brennaria*, and since this genus mainly infects landscape trees, it has not been developed as a model system for the study of phytopathogens.

Although *Pectobacterium* and *Dickeya* are in different genera, they are often discussed together because they produce high levels of plant-cell-wall-degrading enzymes (PCWDE) and cause similar wilt and decay diseases on a wide range of monocot and dicot plant species (Ma et al. 2007). It is the activity of the PCWDE pectate lyase that is used to isolate these genera from plant, water, and soil samples; researchers typically isolate SRE on pectate-containing media, on which the SRE form pitting colonies (Hyman et al. 2001). Since this method can only isolate strains that encode pectate-inducible pectinases, if non-pectolytic members of these

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Table 2.1 *Pectobacterium* and *Dickeya* species

Species	Known host range	References
<i>Dickeya chrysanthemi</i>	Broad host range ^a	Samson et al. (2005)
<i>Dickeya paradisiaca</i>	Banana, maize	Samson et al. (2005)
<i>Dickeya dadantii</i>	Broad host range	Samson et al. (2005), Brady et al. (2012)
<i>Ssp. dadantii</i>		
<i>Ssp. dieffenbachiae</i>		
<i>Dickeya dianthocola</i>	Broad host range	Samson et al. (2005)
<i>Dickeya zeae</i>	Broad host range	Samson et al. (2005)
<i>Pectobacterium aroiderium</i>	Ornamental monocots ^a , potato	Nabhan et al. (2013), Yishay et al. (2008)
<i>Pectobacterium atrosepticum</i>	Potato, pepper, sunflower	Gardan et al. (2003), Hauben et al. (1998)
<i>Pectobacterium betavasculorum</i>	Sugar beet	Gardan et al. (2003), Hauben et al. (1998)
<i>Pectobacterium cacticida</i>	Cacti ^a	Alcorn et al. (1991), Hauben et al. (1998)
<i>Pectobacterium carotovorum</i>		Duarte et al. (2004), Hauben et al. (1998)
<i>ssp. carotovorum</i>	Broad host range among dicots	
<i>ssp. brasiliense</i>	Potato	
<i>ssp. odoriferum</i>	Potato, chicory	
<i>Pectobacterium wasabiae</i>	Wasabi, potato	Gardan et al. (2003), Hauben et al. (1998)

^a Four or more known hosts

genera exist, they will remain uncaptured by standard isolation methods.

Like other *Enterobacteriaceae*, the SRE are gram-negative rod-shaped non-spore forming facultative anaerobes with peritrichous flagella. They are easily grown in culture, and many mutagenesis methods and plasmids developed for the model bacterial species *Escherichia coli* also work with the SRE. Numerous SRE genome sequences are available, and a genial worldwide research community has collaborated on SRE studies for many years (Bell et al. 2004; Glasner et al. 2008, 2011; Nykyri et al. 2012; Pritchard et al. 2013b). Together, these attributes have allowed the SRE to become an important model for phytopathogenesis and a common teaching tool in plant disease laboratory courses.

2.1.2 Agricultural Relevance of *Pectobacterium* and *Dickeya* Genomics

The SRE are widespread and are found in all agricultural regions on numerous fruit, vegetable, and ornamental crops (Ma et al. 2007). SRE species differ in economic significance in

different agricultural regions and on different crops, but the genomic contributions to host range and geographical distribution remain unknown.

Most of the SRE research to date has focused on its impact on potato, which is the most important dicot food crop worldwide. The SRE can cause disease on potato at planting, where they decay seed pieces, during the growing season, where they may kill stems and tubers, and in storage, where they can rot large volumes of potatoes in warehouses or during shipping. On tubers, the disease often starts either from the stem end, where the pathogen often appears to have entered the tuber through the stolon, or from the bud end, where it may have entered due to incomplete periderm formation (Fig. 2.1). The bacteria thrive in these low-oxygen environments, and often, there is a vase-like portion of undecayed tissue surrounding the diseased portion, as though the pathogen was creating an anaerobic chamber inside the tuber. Similar core rots are also seen in carrots infected with SRE (Fig. 2.1). The SRE can infect foliage from infected tubers or from wounds on the stem, and once inside stems, it causes a brown or black decay that can kill the plant (Fig. 2.1).



Fig. 2.1 Typical symptoms of SRE on *potato* and *carrot*. *Top Row* Soft rot in *potato* and *carrot* is often only found in the center of the *tuber* or *root*, highlighting the pathogenesis of these bacteria under anaerobic conditions. *Bottom Row* Chlorotic symptoms are characteristic of *Pectobacterium*, but not *Dickeya*. The *left leaf*

was inoculated via the petiole with *P. carotovorum*, and the *leaf* on the *right* is from an uninoculated control. The SRE cause wilt and decay symptoms on *potato stems*, and they can invade the *leaves* from the *stems* via the petiole. The *plant* on the *left* grew from a *potato tuber* naturally infected with *P. carotovorum*

Pectobacterium also typically causes plants to turn yellow, a symptom that is less apparent in plants infected with *Dickeya* (Fig. 2.1).

There are no curative methods for plants infected with SRE. Copper sprays provide limited control when conditions are conducive for infection, but copper does not affect the bacteria once they have colonized the inside of the plant. Recent studies with sanitizers suggest that treating tubers with bleach or benzoic acid provides useful control (Czajkowski et al. 2013). Thus, sanitation, exclusion, crop rotation, and, to a limited extent, plant resistance remain the most important controls for the SRE (Czajkowski et al. 2011).

The SRE are usually described as brute force broad host range pathogens. However, a phylogenetic analysis of all reported hosts of the SRE initiated several years ago by Arthur Kelman suggests some host specificity among strains and

genera (Ma et al. 2007). For example, *Dickeya* species infect many grain crops (Poales), but there are no reports of *Pectobacterium* attacking major crops such as rice or maize. Similarly, *Pectobacterium* appears to be a significant pathogen on brassica crops, whereas *Dickeya* has not yet been reported on these widely grown crops. Recent phylogenetic work supports this host species analysis. For example, *Pectobacterium* strains that infect monocot ornamentals are genetically distinct from those infecting dicots (Nabhan et al. 2013; Yishay et al. 2008).

Some SRE species have been reported to have narrow host ranges, but the genomic basis for host range in SRE remains obscure. For example, *Pectobacterium wasabiae* had been reported as a wasabi pathogen (Goto and Matsumoto 1987), but sequence analysis of strains from diseased plants later showed that this species is widespread on potato (Baghaee-Ravari et al. 2011;

Moleleki et al. 2013; Pitman et al. 2010). Similarly, *Pectobacterium atrosepticum*, which causes blackleg on potato, is considered a narrow host range pathogen, but it has been reported on crops such as sunflower (Bastas et al. 2009), and it can cause disease on some non-solanaceous plants in controlled environments (Marquez-Villavicencio et al. 2011).

Future work will likely show that like many bacterial plant pathogens, the genera *Pectobacterium* and *Dickeya* have broad host ranges, but individual SRE strains have restricted host ranges. Genomic data have been used recently to develop simple and relatively inexpensive methods to detect and differentiate all SRE species (Pritchard et al. 2013a). However, because home owners or farmers generally do not make control decisions based on SRE species or genus, there is rarely incentive for them to pay for these sophisticated diagnostic tests that could aid in understanding SRE epidemiology.

Because we know very little about how SRE genomes affect regional distribution or host range of these pathogens, we also know little about factors that affect emergence of novel SRE species or strains in agriculture. However, a recent outbreak of a novel *Dickeya* species, *Dickeya solani*, on potato has provided much insight into these questions. *D. solani* was unknown on potato until the past decade, when it emerged swiftly in the Netherlands on potato, and was then spread via seed potatoes to the Middle East, North Africa, and Asia (Degefu et al. 2013; Slawiak et al. 2009; Toth et al. 2011). *D. solani* has now replaced *P. atrosepticum* as the most important SRE on potato in Europe. Unlike *P. atrosepticum* and other well-studied SRE, *D. solani* strains are clonal, suggesting a recent emergence. The available data suggest that *D. solani*, which is also an efficient pathogen of ornamental bulb crops, was spread into potato in the Netherlands due to rotation of potatoes with ornamental bulbs, supporting the hypothesis that crop rotation promotes SRE emergence on new host species. The Netherlands exports large volumes of both ornamental bulbs and potatoes, which resulted in swift dispersal of this pathogen around the world within a few years.

D. solani has not yet been found in the Americas or in South Africa. In these locations, *Pectobacterium carotovorum* subsp. *brasiliense* appears to be more common (Duarte et al. 2004; Ma et al. 2007; van der Merwe et al. 2010). *D. solani* also has not yet been found in some seed-producing regions of Europe. Its emergence in Europe resulted in quarantines in *D. solani*-free regions, such as potato-producing regions in Scotland in an effort to exclude this pathogen (Kerr et al. 2010). In other countries, such as the USA, this pathogen is essentially ignored. Indeed, despite the lessons learned in Europe, potato producers in Idaho have recently begun rotating potato with ornamental bulbs, which is likely to result in a similar spread of novel SRE in North America.

The *D. solani* scenario describes the largest practical impact of SRE genomics on agriculture today. Due to the availability of genomic sequences, researchers were able to quickly identify a swiftly emerging potato pathogen and to develop specific and sensitive diagnostic tools to detect this pathogen (Degefu et al. 2013; Pritchard et al. 2013a). These tools are now being used to maintain *D. solani*-free regions in some seed potato-producing regions of Europe and to understand *D. solani* epidemiology.

2.1.3 Educational Relevance of *Pectobacterium* and *Dickeya* Genomics

A growing human population and climate change increase our need for expertise in food and fiber production, and the need for expertise in plant protection will certainly grow, even as the pool of people available to teach in this area continues to shrink. In a time of shrinking resources, the SRE are useful teaching tools for students interested in learning about bacterial pathogenesis. The freely available SRE genomic resources allow development of laboratory exercises with the bacteria or in silico. Many features of bacterial physiology and pathogenesis, including Koch's postulates, bacterial isolation on selective media, plant cell wall degradation, biofilm

formation, quorum sensing, insect vectoring, bacterial mutagenesis, pigment production, motility, the plant hypersensitive response, autophagy, antibiosis, bacterial iron acquisition, phage responses, comparative genomic analyses, and contact-dependent inhibition (CDI), can be demonstrated with the SRE. They grow quickly and cause dramatic plant symptoms, and because they do not infect vertebrates, these genera are among the safest bacteria for students to work with. They are also ubiquitous, so teachers can easily obtain local isolates, eliminating the need for maintenance or transfer permits. Finally, the development of ASAP (Glasner et al. 2003), which is free intuitive peer-reviewed database that allows students to enter new findings about SRE genomes, provides a way for undergraduate and graduate students to learn about genomics and, at the same time, to contribute to phyto-bacteriological research (Glasner et al. 2006).

2.1.4 The SRE Life Cycle

The SRE can cause disease at all stages of production, from planting through storage. Plants may become infected or contaminated with SRE at any point in the production cycle. The SRE are found in irrigation water, soil, and on insects and can latently colonize plants, so it is difficult to keep plants free of SRE in greenhouses or in the field. Fortunately, the SRE are not as aggressive as some viral, fungal, and oomycete pathogens, so complete losses due to SRE during the growing season are rare. However, even relatively, small losses can complicate crop management. For example, poor plant emergence in a potato field due to soft rot of seed tubers makes the potato crop difficult to manage since the plants adjacent to the rotted tubers will grow larger and the harvested tubers will have an uneven size profile. The SRE appear to be significant only in agricultural environments since epidemics in natural environments have not been reported, unlike, for example, as occurs with some fungal tree diseases, such as Dutch elm disease or chestnut blight.

The most significant SRE-caused losses are in storage, where the bacteria from a few decaying vegetables can quickly spread to entire piles of stored vegetables. Unlike many bacterial pathogens, the SRE are facultative anaerobes, and some of their virulence genes are upregulated under anaerobic conditions (Babujee et al. 2012; James and Hugouvieux-Cotte-Pattat 1996). This may explain why they can devastate stored vegetables, where the rotting piles quickly become anaerobic. The high virulence of these strains under anaerobic conditions is often evident in symptom development in vegetables, which are often only rotten in the center, with the ring of tissue outside of the vascular ring remaining undecayed (Fig. 2.1).

The SRE life cycle was once considered to be very simple, and SRE were described as brute force pathogens, with the bacteria colonizing wounded or stressed plants and decaying them through the action of PCWDE. A major impact of genomic studies on the SRE has been a greater appreciation for the subtleties of SRE interactions with plants, insects, and other microbes (Costechareyre et al. 2010, 2013; Llama-Palacios et al. 2002; Toth and Birch 2005). The brute force view of the SRE life cycle is likely the major reason that more effort has not been put into understanding plant resistance over the past century. A long-term impact of genomic research will likely be an increased appreciation for multiple mechanisms required for SRE pathogenicity and resurgence in studying plant resistance to the SRE.

2.1.5 Will Insights from SRE Genomes Lead to Disease Control?

The genetics of SRE pathogenesis were first explored in the early 1970s (Beraha and Garber 1971; Beraha et al. 1974; Chatterjee and Starr 1972), and hundreds of genetic and genomic articles about SRE pathogenesis have been published since then. Despite this, we still use the same control recommendations that farmers have been using for generations—sanitation,

exclusion, crop rotation, and resistance. Many possibilities for disease control that were fostered by genomic studies remain on the horizon, either because the methods proposed, such as transgenic plants, are expensive to implement and not acceptable to consumers or because we still lack detailed information needed about plant and insect interactions with the SRE that could lead to useful control methods. As described above, the most significant contribution today from SRE genomics is an improved ability to detect and differentiate SRE taxa. The most significant contribution to control on the horizon will likely be improvements in breeding for SRE resistance and in control of insect vectors of the SRE.

2.2 Recent Insights into Pathogenesis Made from SRE Genomics

More *Enterobacteriaceae* species genomes are available than from any other family of organisms. The numerous *Enterobacteriaceae* and SRE genomes available have allowed useful applications of genomic data in control and treatment of diseases caused by this important bacterial family. However, much remains to be done in development of new controls, understanding the evolution and pathogenesis in this bacterial family, and exploring the ecology of these species.

P. atrosepticum SCRI 1043 was the first SRE genome sequenced (Bell et al. 2004). The *Dickeya dadantii* 3937 genome was sequenced at approximately the same time as a large community project (Glasner et al. 2011). Although the progress toward publication of the 3937 genome was slow, the collaborative nature of the project and associated genome meetings were effective at building rapport among this research community. In recent years, numerous additional *Pectobacterium* and *Dickeya* genomes have been sequenced by individual lab groups and deposited in public databases (Glasner et al. 2008, 2011; Nykyri et al. 2012; Pritchard et al. 2013b). These genome sequences have aided in

resolving phylogenetic relationships within genera, but, perhaps due to high rates of horizontal gene transfer, it has been difficult to determine how the SRE and related genera are placed within the *Enterobacteriaceae* tree.

The SRE genomes consist of a single circular chromosome of just under 5 Mb in size. None of the sequenced strains have sequenced plasmids, although small plasmids and other types of extrachromosomal elements have been reported in the SRE (Nomura et al. 1996). Like many *Enterobacteriaceae* species, the *Pectobacterium* genome is largely conserved, with indels accounting for much of the genome differences among species (Bell et al. 2004; Glasner et al. 2008). Similarly, the *Dickeya* species backbone is also largely conserved (Glasner et al. 2011; Nykyri et al. 2012; Pritchard et al. 2013b). For the most part, the contribution of the various indels to SRE pathogenicity remains unknown, although there are several examples where the genes encoded by these regions contribute to virulence (Evans et al. 2010; Glasner et al. 2008; Hommais et al. 2008; Nykyri et al. 2012, 2013; Pérez-Mendoza et al. 2011; Williamson et al. 2010). At least some of these indels are able to excise from the genome and replicate extra-chromosomally (Vanga et al. 2012).

Much of the SRE genome-enabled research over the past few years has focused on identification and characterization of the roles of these indels to SRE virulence. Through this work, we have learned about SRE adhesion (Jahn et al. 2011; Pérez-Mendoza et al. 2011), pigments (Williamson et al. 2010), potential toxins (Bell et al. 2004), contact-dependent secretion (Aoki et al. 2010), and even novel plant-cell-wall-degrading enzymes (Rondelet and Condemine 2012). The SRE genome sequences have also led to insights into insect vectoring, and infection by SRE and the realization that genes required for insect interactions are among the least conserved portions of the SRE genomes (Acosta Muniz et al. 2007; Basset et al. 2003; Costechareyre et al. 2010, 2013; Quevillon-Cheruel et al. 2009).

SRE genomes have enabled a limited number of gene expression studies via promoter-identification studies, microarrays, or chromatin

immunoprecipitation microarrays. Most of these gene expression experiments were designed to catalog genes controlled by well-known central regulators, and the essential discovery was that most of these regulators control large numbers of virulence and central metabolism genes (Hommais et al. 2008; Koiv et al. 2013; Liu et al. 2008; Monson et al. 2013; Rodionov et al. 2004; Venkatesh et al. 2006; Yap et al. 2008). A contrast to this is an assessment of genes controlled by the type III secretion system sigma factor HrpL in *Pectobacterium*, where HrpL was found to control mainly the type III secretion system (T3SS) and genes encoding harpins and a single known effector, DspE (Hogan et al. 2013), rather than being integrated into control of a wide range of other virulence or central metabolism genes.

A few SRE gene expression studies have focused on conditions experienced by SRE and provided insights into bacterial mechanisms used to survive under diverse stresses, such as survival inside insects or during a shift from aerobic to anaerobic conditions. These experiments led to novel insights about the SRE and their host plants or insects. For example, *D. dadantii* upregulates genes required for detoxifying antimicrobial peptides, an antibiosis mechanism not previously thought to be important for the aphid immune system (Costechareyre et al. 2013). Antimicrobial peptides have long been known to be important for plant defenses, but only recently, through gene expression analyses, have we learned that SRE responses to these peptides are controlled via the PhoPQ two-component system (Rio-Alvarez et al. 2012). An investigation into SRE responses to oxygen availability clearly showed that although *Pectobacterium* and *Dickeya* share many regulatory genes, their response to a shift to low-oxygen conditions differed (Babujee et al. 2012), with *Pectobacterium* being better suited to growing in low-oxygen conditions. These data also supported a theme seen many times in comparisons between these two genera; although they cause similar symptoms, the regulatory pathways used to control similar virulence genes differ among *Pectobacterium* and *Dickeya* [for details

see (Charkowski et al. 2012)]. Thus, conclusions about regulatory networks cannot necessarily be generalized across the SRE.

2.2.1 Genomics and Secretion

Genome sequences revealed that the SRE, as a group, encode six of the seven protein secretion systems known in gram-negative bacteria (Fig. 2.2), and their role in virulence was recently reviewed (Charkowski et al. 2012). Because several of these secretion systems play key roles in virulence, they have been explored with multiple post-genomic methods, such as gene expression arrays and proteomic surveys. The SRE also secrete or produce extracellular polysaccharides that play important roles in biofilm formation and diseases.

T1SS. The SRE strains encode type I secretion systems (T1SS), which they likely use to secrete proteases and adhesins (Pérez-Mendoza et al. 2011). With the exception of the single report by Pérez-Mendoza et al. (2011), these systems have been almost completely unexamined in the past decade.

T2SS. All SRE encode a type II secretion system, which secretes PCWDE, such as pectinases and cellulases, as well as other virulence-related proteins. Secretome analyses with SRE have focused in large part on proteins that travel the T2SS (Kazemi-Pour et al. 2004). The action of the pectinases is required for isolation of SRE on pectate-containing media, so due to the isolation method used, the presence of these genes in SRE is expected. Some SRE encode a second T2SS, which has only one known target, a cell-bound pectin lyase (Ferrandez and Condemine 2008).

Hrp T3SS. Many, but not all, SRE encode a Hrp T3SS, which appears to secrete a single effector protein, DspE (Hogan et al. 2013). Genome sequences were instrumental in demonstrating that one or, at most, only a few effectors travel the T3SS. How this single effector promotes virulence remains unknown; it plays no apparent role in suppressing plant

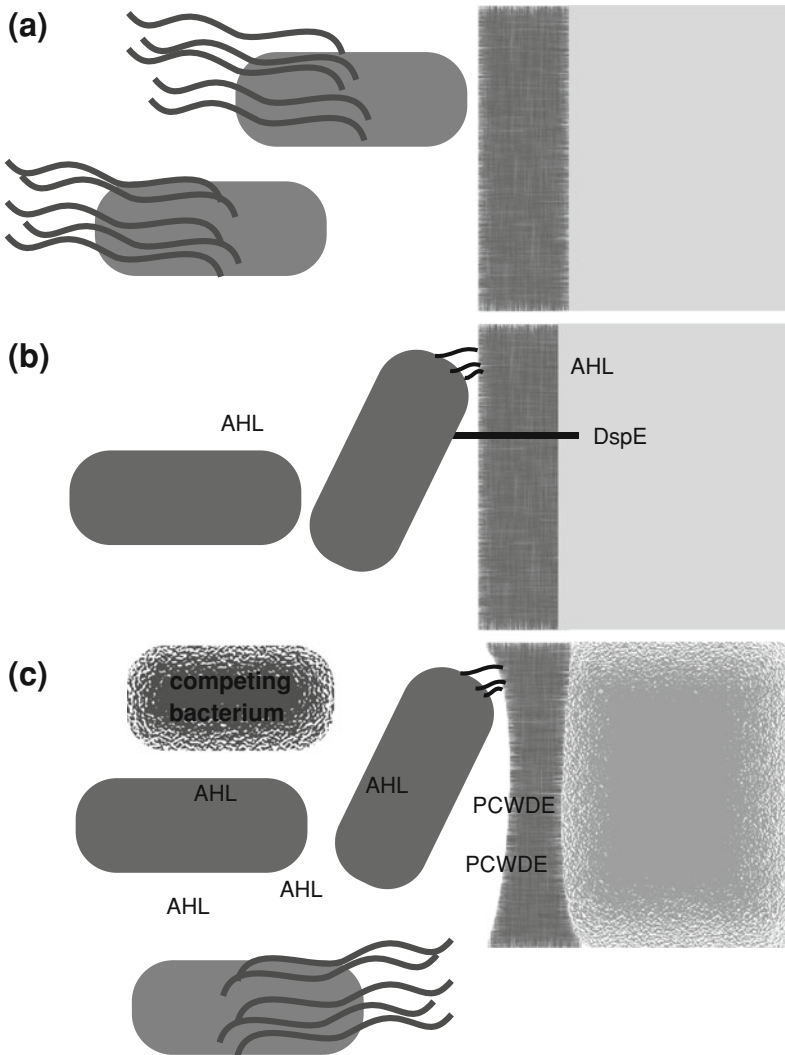


Fig. 2.2 SRE infection of plant tissue. **a** The SRE have flagella and use chemotaxis to move toward wounded plant tissue. **b** Once adjacent to plant cells, they adhere to the cells with secreted adhesin proteins. Some SRE elicit plant cell death by translocating their single known T3SS effector, DspE, into plant cells. **c** They sense that they are in plant cells by integrating signals, such as bacterial AHL and plant cell wall fragments, and then produce massive amounts of plant-cell-wall-degrading enzymes (PCWDE) to macerate plant cell walls. The SRE encode

multiple antimicrobial systems and can kill other bacterial strains and species with antibiotics, the contact-dependent inhibition system, or bacteriocins. The SRE are motile in macerated lesions. They multiply and spread from the initial infection zone both locally from cell to cell and systemically via the plant vascular system. Multiple cell types per lesion (e.g., motile and sessile) and cell motility complicate gene expression analysis and growth analysis of SRE in infected plants

defenses. Among the SRE, *P. wasabiae* is notable for its lack of a Hrp T3SS, although strains in other clades also lack this system (Kim et al. 2009; Nykyri et al. 2012; Pitman et al. 2010). Some T3SS contribute to bacterial

aggregation in culture; this phenotype for the T3SS was first noted with *Dickeya* (Yap et al. 2005).

Flagellar T3SS. The SRE are motile via a flagellar T3SS. Multiple flagellins are commonly

encoded by SRE genomes, but whether the different flagellins affect virulence remains unknown. Unlike many plant pathogens, the SRE are often motile in diseased plants. The flagellar regulator FliA is required for *Dickeya* virulence (Jahn et al. 2008), but the contribution of motility to virulence has been almost unexplored in our post-genomic era. Swimming may play a role in addition to motility since SRE swimming increases the efficacy of PCWDE or signaling by mixing enzymes or signaling compounds in decaying plant material.

T4SS. The type IV secretion system is only encoded by some strains, and its targets and role in SRE virulence remain unclear (Bell et al. 2004; Glasner et al. 2008; Nykyri et al. 2012).

T5SS. Compared to the other secretion systems in the SRE, the type V secretion system is little explored. It appears to contribute to *Dickeya* adherence and aggregation during pathogenicity (Rojas et al. 2002).

T6SS. The SRE also encode type VI secretion systems, but their role in SRE biology remains unclear (Nykyri et al. 2012).

Pili. The SRE encode pili genes, but they have been little examined, and their role in disease remains unknown (Nykyri et al. 2013).

Cellulose. Bacterial cellulose is used for biofilm formation by many genera. The SRE encode two different types of cellulose synthesis operons, with *Pectobacterium* encoding synthesis genes commonly found in other *Enterobacteriaceae* and *Dickeya* encoding a cellulose synthesis gene cluster that appears to have been recently horizontally acquired. The cellulose synthesis cluster in *Dickeya* contributes to biofilm formation (Jahn et al. 2011), but makes no known contribution to virulence.

2.2.2 Bacterial Signal Molecules and Disease Control

Disruption of virulence gene expression is an attractive mechanism for control of diseases caused by the SRE. *Pectobacterium* and *Dickeya* regulate virulence genes by integrating responses to inter- and intracellular bacterial signals

and to plant signals [recently reviewed in Charkowski et al. (2012)]. Although the symptoms caused by these two genera are similar and their main pathogenicity factor is production of a massive amount of PCWDE, regulation of these enzymes, including the signals that the bacteria produce and respond to, has diverged within the SRE.

In both genera, acyl-homoserine lactone (AHL)-mediated quorum sensing plays a role in virulence gene activation, although its importance varies greatly among strains (Ham et al. 2004; Hussain et al. 2008; Mhedbi-Hajri et al. 2011). *Pectobacterium* was the first pathogen in which AHL-mediated quorum sensing was shown to control virulence genes (Pirhonen et al. 1993) and AHL appears to be universally important in this genus. In *Pectobacterium*, AHL-mediated quorum sensing regulates approximately one-quarter of the genes through its regulation of the RsmA-*rsmB* RNA degradation pathway (Chatterjee et al. 2002; Liu et al. 2008). When AHL binds to the DNA-binding proteins ExpRI or ExpR2, it reduces the affinity of ExpR for DNA, resulting in decreased RsmA production (Cui et al. 2005, 2006). Since RsmA targets virulence protein mRNAs for degradation, less RsmA results in higher production of some proteins, including many virulence genes (Chatterjee et al. 1995; Mukherjee et al. 1996).

Genome sequence-enabled studies have resulted in identification of additional bacterial signal molecules in the SRE. These intracellular signaling molecules are widespread among bacteria, such as the autoinducer 2 (AI2) system and cyclic-di-GMP (c-di-GMP)-mediated signaling. Both *Pectobacterium* and *Dickeya* encode *luxS*, which is required for production of the AI2 signal (Crepin et al. 2012), but the role this signal plays in the SRE life cycle remains unknown. The c-di-GMP system is an example of an intracellular signaling system that was revealed in large part through genomic data (Ryan et al. 2012). In bacteria, enzymatic modification of c-di-GMP is involved in cell differentiation in response to environmental or physiological cues. Regulation may occur through sensing of c-di-GMP levels, which are

altered by diguanylate cyclases or phosphodiesterases, or via riboswitches upstream of key genes required for adhesion or motility (Sudarsan et al. 2008). Recently, seven of the 18 proteins in these classes were mutated in *D. dadantii*. Two of the mutant strains (*ecpB* and *ecpC*) had enhanced biofilm formation and reduced virulence, motility, pectate lyase production, and T3SS gene expression (Yi et al. 2010). These same two genes also contribute to virulence in *Pectobacterium*, where they regulate motility and expression of a T1SS, which secretes an adhesin (Pérez-Mendoza et al. 2011). Since these intracellular signaling systems are widespread in both plant and animal pathogens, they are unlikely to be useful targets for disease control in agriculture.

SRE genomics enabled discovery of less conserved signaling systems in *Dickeya*, including the Vfm system and a putative auxin-mediated system. At least, some *Dickeya* strains appear to produce auxin, and it may be a crucial signaling molecule since mutants unable to produce auxin are non-pathogenic (Yang et al. 2007). The auxin signaling pathway acts via the RsmA-*rsmB* pathway, but how auxin is sensed by *Dickeya* remains unknown. Like auxin, the Vfm pathway is only present in *Dickeya* and not *Pectobacterium* (Nasser et al. 2013). The 25 kb Vfm locus, which is located adjacent to the AHL locus encoding ExpR and ExpI in *D. dadantii*, is required for PCWDE production by this pathogen. This locus produces a signal that regulates both virulence gene expression and expression of Vfm locus genes, reminiscent of the feedback regulation of AHL-mediated quorum sensing. These regulatory systems, which appear to be specific to *Dickeya*, may be useful targets for control of this genus.

The key role of bacterial signaling in virulence makes these systems obvious targets for disruption of pathogenicity, but implementation of these methods in agriculture has not yet occurred. To date, the only exploration of signal disruption has been with AHL-mediated signaling. Researchers have examined the use of AHL antagonists and of plants that degrade or produce AHL. Regardless of their efficacy in culture,

AHL antagonists are unlikely to be effective under most field conditions since there is a short window of time during infection that these compounds are effective at disrupting pathogenicity (Palmer et al. 2011). However, it is possible that such compounds could be useful under very specific conditions, such as treating plant cuttings just prior to planting.

Transgenic plants that produce an AHL lactonase derived from *Bacillus*, AiiA, are able to constantly degrade AHL and thereby resist soft rot caused by *Pectobacterium* (Dong et al. 2000, 2001). As expected, transgenic potato plants that produce AHL through targeting an AHL synthase to the plant chloroplast are more susceptible to tuber soft rot and stem decay (Toth et al. 2004), further supporting the importance of AHL to SRE pathogenicity. Although plants that disrupt AHL signaling resist bacterial soft rot and AHL signaling is common among rhizosphere bacteria, these plants do not have detectable effects on bacterial colonization of roots (D'Angelo-Picard et al. 2011; Dong et al. 2000, 2001).

Similar future signal disruption experiments with antagonists or transgenic plants will likely provide important information about the various bacterial signaling systems important for virulence. However, transgenic plants that target any particular signaling system may not provide long-term control of SRE diseases since these plants would likely select for strains that use alternate signaling pathways.

2.2.3 Genomics, Bacterial Metabolism, and Disease Control

Multiple intracellular metabolites aid in integration of information about bacterial environment and physiology and thereby play key roles in SRE gene expression during pathogenicity. Unfortunately, for the most part, large holes remain in our knowledge of SRE metabolism. Of those signals that are known, most have only been studied individually and not as part of a comprehensive metabolic model. Although novel control methods have not yet been developed based upon this

metabolic information, these results help us explain how some of our current control methods work.

To date, glucose metabolism has been most closely examined. Like typical *Enterobacteriaceae*, the SRE grow efficiently on glucose and repress other metabolic pathways via the cyclic AMP receptor protein, CRP, including the production of enzymes required to digest plant cell walls, when glucose is present (Reverchon et al. 1997). In turn, KdgR, a repressor that regulates pectinases and other virulence genes also regulates gluconeogenic enzymes (Rodionov et al. 2004), providing a mechanism to coordinate metabolism and plant cell wall degradation. There are several other hints that central metabolism is closely tied to virulence. For example, the starvation signal (p)ppGpp is required for PCWDE production (Wang et al. 2007). Similarly, mutation of gluconate metabolic genes results in hyper-maceration, a lack of motility, and mis-regulation of KdgR and the flagellar regulator FlhD (Mole et al. 2010). Genomics will undoubtedly provide new insights into ties between metabolism and virulence now that we have a more comprehensive inventory of the metabolic pathways present in the SRE and tools to examine expression of metabolic pathway genes in response to different stimuli. As an example, Babujee et al. (2012) recently reported the numerous and divergent effects that a shift to low-oxygen conditions has on *Pectobacterium* and *Dickeya* gene expression, demonstrating that conserved metabolic pathways may respond differently to the same stimulus even in closely related genera.

A comprehensive metabolic model for the SRE will also likely provide insights into host range and the rationale for the effects of various fertilizers on SRE-caused diseases. For example, recent observations that phosphorus increases susceptibility of calla lilies to soft rot may be tied to the effects of the PhoPQ regulon on virulence gene expression, providing an example of how genomic studies combined with greenhouse experiments could lead to useful recommendations for farmers (Gracia-Garza et al. 2004; Llama-Palacios et al. 2003, 2005).

2.2.4 Genomics, Plant Signal Molecules, and Disease Control

The SRE have long been known to activate virulence genes in response to plant cell wall fragments, but until recently, little else was known about how they perceived plant cells and respond to plant cell signals. An exciting recent finding aided by genomics is the discovery that *D. dadantii* is attracted to and swims toward the wound hormone, jasmonic acid (Antunez-Lamas et al. 2009). The SRE encode numerous methyl-accepting chemotaxis (Mcp) receptors, which are membrane proteins that allow bacterial cells to perceive and move along a chemical gradient. The identity of the Mcp that senses jasmonic acid remains unknown as do the targets of nearly all of the over 40 different Mcp proteins encoded by the SRE.

The SRE also sense other organic acids commonly produced by plants. For example, *o*-coumaric acid and *t*-cinnamic acid, both of which are intermediates in the salicylic acid biosynthesis pathway of plants, activate the *D. dadantii* T3SS (Yang et al. 2008), suggesting that regulators controlling the T3SS sense plant-derived chemicals. There are likely to be many additional plant-produced signals waiting to be discovered, and some of these may explain differences in host susceptibility to the SRE.

2.2.5 Genomics, Microbial Antibiosis, and Disease Control

Antagonism between two microorganisms may be based on one or more mechanisms such as nutrient competitiveness, secretion of growth inhibitory compounds, and antibiotic production or through inducing systemic resistance in plants. Several SRE genes dedicated to compete with other microbes were known before any SRE genomes were sequenced, but SRE genome sequence analysis highlighted that much of the variable portion of SRE genomes is dedicated to producing or protecting against antimicrobial metabolites, proteins, or phage (Bell et al. 2004; Glasner et al. 2008).

The systems studied to date in the SRE include antibiotic production (McGowan et al. 2005), bacteriocins (Grinter et al. 2012; Roh et al. 2010; Tovkach 1998), iron competition (Expert 1999), export of bacterial antimicrobial compounds (Llama-Palacios et al. 2002), and the contact-dependent inhibition systems (Aoki et al. 2010). Few of these systems have been evaluated in greenhouse or field experiments, and global studies of the contribution of an entire SRE genome to microbial competition or of the evolution of genes involved in microbial competition have not yet been done.

Among the most exciting new fundamental discoveries in antibiosis studies derived from genomics is the discovery of the CDI system. Recently, the SRE pathogen *D. dadantii* was used to demonstrate that Rhs proteins and related YD-peptide repeat proteins, which are present in a wide range of bacterial species, including other SRE, inhibit growth of neighboring bacteria. Rhs carry polymorphic C-terminal toxin domains which are predicted to be deployed into target cells using the type VI secretion system. *D. dadantii* 3937 is able to protect itself from the cognate toxins and auto-inhibition by encoding sequence-diverse immunity proteins (RhsIB) (Aoki et al. 2010; Koskiniemi et al. 2013; Poole et al. 2011). Since the CDI system is usually only functional when the bacteria are in association with host cells and SRE virulence assays are simpler and less expensive than model pathogens that require animals, the SRE will likely continue to be an important model system for studying CDI system function.

The most likely avenue for disease control via antibiosis is through phage therapy. Phage therapy trials with *Pectobacterium* have been conducted on calla lily tubers in greenhouse settings and demonstrated to reduce disease incidence by up to fifty percent (Ravensdale et al. 2007). Recently, two phages, vB_DsoM_LIMEstone1 and vB_DsoM_LIMEstone2, were isolated and characterized for the control of *D. solani*, an aggressive biovar 3 variant of *Dickeya dianthicola* (Adriaenssens et al. 2013). In this case, phage therapy reduced disease incidence and

severity in greenhouse trials with inoculated potatoes and resulted in higher yields in potato field trials. The species within the SRE are diverse, and multiple taxa are commonly found in fields (Kim et al. 2009; Yap et al. 2004), and thus, treatments with individual phage are unlikely to be successful in most cases. However, increased efficiency of phage therapy may be achieved through the use of phage cocktails containing multiple phage types (Adriaenssens et al. 2013; Jones et al. 2007). In the future, genomic-enabled detection methods and phage analysis may allow us to assess an agricultural environment or seed lot and determine the appropriate phage cocktail for SRE disease control.

2.2.6 Plant Resistance and Disease Control

Although some examples of single dominant genes conferring resistance to SRE have been known for decades (Lewellen et al. 1978; Whitney and Lewellen 1978), the mechanisms of resistance to soft rot disease remain mostly unknown and were little studied until recently. Data from both cultivated and wild potato suggest that pre-formed resistances occur such as differences in plant cell walls, protease inhibitors, antimicrobial peptides, or other antimicrobial compounds. These genes may appear as quantitative trait loci in genetic experiments designed to identify SRE resistance genes.

Much of what we know about plant resistance to bacterial pathogens is derived from *Pseudomonas syringae*—*Arabidopsis* interactions or *Xanthomonas*—rice interactions. Unfortunately, these hemibiotrophic pathogens use very different virulence strategies. Consequently, models based on these pathosystems provide little insight into resistance to SRE. Similarly, many pathosystems examined in *Arabidopsis* use a few key hormones, namely salicylic acid, jasmonic acid, and ethylene for defense pathway signaling. But again these systems may not play an important role in resistances observed in some plant species or varieties in the field and

greenhouse. Also, plant disease resistance has mainly been studied with leaf pathogens. The SRE are stem, root, and tuber colonizers, so models based on foliar pathogens that colonize leaf surfaces may only be tangentially useful for understanding SRE pathogenesis.

Only a few researchers have attempted to map SRE resistance in plants, and this work has mainly been done with *P. atrosepticum*. In potato, there are genes that contribute to leaf or tuber resistance to *P. atrosepticum* on all 12 chromosomes (Zimnoch-Guzowska et al. 2000). The available data suggest that leaf and tuber resistance is independent, highlighting the complexity of studying resistance to this pathogen (Zimnoch-Guzowska et al. 2000). Whether the same genes provide resistance to other *Pectobacterium* species or to *Dickeya* is unknown.

Wild species likely encode novel SRE resistance genes. In an assessment of tubers from 123 accessions of wild potato species, *P. carotovorum*-resistant plants were found, but there was no tie to potato taxonomy or strong ties to biogeography of the accessions (Chung et al. 2011). Despite the difficulty in predicting where to find resistant wild species, these results show that novel resistance genes are available in wild relatives of an important crop species.

2.2.7 Genomics Contributions to Pathogen Detection and Epidemiology

The field of study that most separates plant pathology from other research focused on microbial symbiosis is pathogen epidemiology. Until recently, nearly all epidemiological work on the SRE focused on *Pectobacterium* in potatoes grown in temperate climates. Almost nothing is known about the epidemiology of the SRE in tropical and subtropical climates. In the past few years, considerable work has focused on the epidemiology of *D. solani* due to its emergence as an important potato pathogen in Europe. We know that the SRE can be found in irrigation water, agricultural soils, insects, snails, and in latent associations with the roots of

numerous plant species. They are not typically found on leaf surfaces nor do they tend to cause diseases of trees or emergence problems with plants grown from true seed. Even on inoculated potato in field trials, SRE disease is sporadic, suggesting that much remains to be learned about environmental components of SRE–plant interactions.

One of the most significant contributions now used to control SRE can be tied to disruption of SRE epidemiology, and specifically in disruption of insect vectoring of SRE. The reduction of *Dickeya* stalk rot in maize as a result of transgenic insect-resistant plants is a significant example of control through disruption of the vector life cycle (Dalmacio et al. 2007). Maize stalk rot used to be a significant concern in North America (Thind and Payak 1976; Thind and Singh 1976), but no work has been published from North America on this disease in decades. The reason for the quiet disappearance of this disease from the literature is likely due to a combination of the prevalence of insect-resistant transgenic maize, the lack of phyto bacteriologists working on SRE diseases in North America, the almost complete lack of routine survey data for most plant diseases, including all SRE diseases, and the nearly complete control of research and maize seed production by a handful of large corporations.

Although reports of SRE–insect associations go back over 80 years (Leach 1926, 1931, 1933), very little is known about SRE–insect interactions. However, this has begun to change, and it is one of the largest contributions of SRE genomics toward understanding of SRE ecology and epidemiology. The relationship between SRE and insects is complex, with evidence for insects acting as SRE vectors (Molina et al. 1974) and for SRE being a deadly insect pathogen (Costechareyre et al. 2010). Prior to availability of SRE genome sequences, the gene, *Evf*, which contributes to *Pectobacterium* fitness on fruit flies (*Drosophila melanogaster*), was identified (Basset et al. 2003). *Evf* is a lipid-binding protein that requires palmitoylation for function, but how it contributes to bacterial colonization of fly guts remains unknown (Quevillon-Cheruel

et al. 2009). Although *Evf* is required for *Pectobacterium* colonization of fruit flies, it is not conserved among *Pectobacterium* strains. Whether only a subset of *Pectobacterium* colonizes fruit flies or whether another gene has an analogous role in colonization remains unknown.

The effect of *Dickeya* on insects is much more dramatic since some strains infect and kill aphids. A locus encoding four tandem repeats of a cytotoxin and possibly the ability to detoxify antimicrobial peptides contribute to *D. dadantii* insect pathogenicity (Costechareyre et al. 2010, 2013). *D. dadantii* has a limited host range on insects, and its virulence is affected by aphid life stage, suggesting an intricate interaction between these two organisms. Like *Evf*, the *Dickeya* cytotoxin genes are not widespread in the SRE, making it clear that we have much to learn about the diversity of SRE–insect interactions and their impact on SRE epidemiology.

A third set of genes likely to be involved in SRE–insect interactions is widespread in the SRE and in many other bacteria. The action of these genes, detected through the Voges–Proskauer assay, has been used to differentiate environmental *Enterobacteriaceae* from coliform *Enterobacteriaceae* for decades (Levine 1916). The potential role of this gene cluster, denoted as *budAB*, in insect interactions was brought to the forefront by genomic studies. The *budAB* operon encodes two of the three enzymes required for the 3-hydroxy-2-butanone (3H2B) pathway, which is an alternate fermentation pathway (Huang et al. 1999; Lopez et al. 1975). This operon is expressed in plants and required for virulence, particularly under anaerobic conditions (Effantin et al. 2011; Marquez-Villavicencio et al. 2011). There are two possible volatile compounds produced by this pathway, 3H2B, and the related compound 2,3-butanediol (23B). Both can function as kairomones for a wide variety of insects, including sap beetles (*Carpophilus huerfalis*) (Nout and Bartelt 1998), lygus bugs (*Lygus* sp.) (Buttery et al. 1984), cockroaches (*Nauphoeta cinerea*) (Moore and Moore 1999; Moore et al. 2002), Melanesian rhinoceros beetles (*Scapanes australis*) (Rochat et al. 2002), sorghum chafers (*Pachnoda interrupta*) (Bengtsson et al. 2009),

and Mexican fruit flies (*Anastrepha ludens*) (Robacker and Lauzon 2002). The mechanism for attraction has only been described for fruit flies, to date. These insects normally avoid CO₂, which is emitted by ripening fruit, but 23B inhibits antenna neurons sensitive to CO₂ (Turner and Ray 2009). Fruit flies and other insects are often found in and around decaying fruit and vegetables, with massive numbers of fruit flies commonly found in warehouses when soft rot symptoms develop on stored vegetables. Whether the volatile molecules produced by the *budAB* operon are part of what attracts insects to decaying plants in warehouses, compost piles, and kitchens remains unknown.

2.3 Future Prospects for Disease Control Simplified by Genomics

Scientists studying SRE spent the past several decades focused on the genetics, and later the genomics, of SRE–plant interactions and have learned a tremendous amount about how this pathogen deconstructs plant cells. Concurrently, epidemiological and phylogenetic studies have clarified where SRE are in agricultural environments and how these species are related to each other and to other *Enterobacteriaceae*. And yet, we have made little progress in control of diseases caused by SRE. The largest recent contributions toward disease control have been in testing methods that allow faster disease diagnosis and in the serendipitous effects of the BT transgene in maize in essentially eliminating *Dickeya* stalk rot.

The SRE are closely related to *E. coli* and *Salmonella*, two animal pathogens that are very well characterized by a large research community. Despite our large body of genomic knowledge for these human pathogens, we still use antibiotics to treat diseases caused by these microbes, just as we have for the past 70 years. This suggests that significant breakthroughs in chemistry, metabolism, or signaling must be made before bacterial genomic studies lead to control of SRE diseases through manipulation of the bacteria. However, a combination of

information from SRE and host plant genomics is likely to lead to clearer understanding of how some plant species resist SRE; this work will likely lead to control through plant resistance in the near future. Importantly, SRE-resistant plants represent a sustainable, environmentally friendly disease control approach, and breeding and deployment of resistant plants does not require significant technological or theoretical breakthroughs. But it does require sustained funding for plant breeding and plant–microbe interaction studies.

2.3.1 Development of SRE-Resistant Plants

Those attempting to control plant pathogens have one large advantage that researchers studying human *Enterobacteriaceae* pathogens, such as *E. coli*, lack. We are able to breed the host for disease resistance. Although plant resistance is always among the best options for disease control, scientists have focused very little effort on understanding resistance to the SRE. One reason for this lack of research is that the SRE attack mainly vegetable and ornamental crops, and SRE resistance is often not the most important characteristic to breed for. Also, compared to major food crops, little is known about the genomics of these plants. However, this is quickly changing, and genome sequences of numerous vegetable crop plants are now available. In addition, researchers have defined the SRE as brute force secondary pathogens for which genetic resistance might not be available, and therefore, many researchers simply have not searched for it. However, SRE-resistant wild relatives of major vegetable crops are fairly easy to identify. Recently available genomic tools should allow identification of the types of genes that confer resistance to SRE.

Potato, one of the major hosts of the SRE, is a good example of underutilized SRE-resistant germplasm and availability of genomic tools. Unlike most other SRE hosts, potato is a major food crop with an active worldwide research community. But, unlike other major crops, such

as maize, soybean, and canola, there is relatively little corporate interest in potato; this crop remains mostly in the hands of family farms and public researchers. Thus, there has been little investment in potato genomics compared to maize and soybean, and until very recently, no genome sequence was available (Xu et al. 2011). Commercial lines of potato are tetraploid, which makes them difficult to sequence and to assess through standard genetic methods. Of necessity, sequencing efforts initially focused on an inbred potato that bears little resemblance to commercial potato. Essentially, no disease resistance work has been completed by the larger research community with this sequenced line.

Fortunately, the potato genus, *Solanum*, is highly diverse, and many species within this genus are easily hybridized. In addition, discoveries in potato genetics now allow fairly simple breeding of diploid potato capable of self-fertilization, which allows development of inbred lines more similar to commercial potato and suitable for disease resistance studies (Lindhout et al. 2011). Since there are multiple wild *Solanum* species that are resistant to SRE, this line of work holds promise both for understanding how plants resist the SRE and for development of SRE-resistant varieties.

2.3.2 Insect Control

SRE genomics has provided new insights into the epidemiology and ecology of these pathogens, and as a result, it has drawn new researchers to this field. For example, SRE–insect interactions have been sporadically studied for the past 80 years, but the genetics and epidemiology of these relationships remain little characterized. Recently, decoded genomes of SRE and other plant pathogenic *Enterobacteriaceae* have clarified that insects play a large role in dissemination of these pathogens and suggest that several of these genera have intimate interactions with insects. Control of insects through transgenic plants has essentially eliminated SRE disease in maize, suggesting that insect resistance would benefit other crops as

well. It is possible that modern synthetic pesticides have also reduced SRE diseases, but we have almost no historical or current survey data for SRE disease incidence on any crop.

Despite the apparent relationship with insect vectors, SRE genes known to be involved in insect interactions are not well-conserved among SRE species or strains, suggesting that conserved genes required for insect interactions remain uncharacterized and/or that only some SRE strains are vectored by insects. Identification of major potential insect SRE vectors and development of systems to study these vectors would aid in determining if vector control would reduce SRE incidence in vegetable and ornamental crops.

2.3.3 Antibiosis

These methods are likely to be based on species- or strain-specific antibiosis techniques that remain almost entirely unexplored at the farm level, such as phage-mediated control or contact-dependent inhibition. If simple detection methods could be followed up by targeted control through phage or other similar methods in seed lots, soils, or water supplies known to carry aggressive SRE strains, a useful level of SRE control might be achieved. The accurate and sensitive diagnostic tools that allow researchers to differentiate among SRE species and strains in order to study their epidemiology in detail have recently been developed, so one factor required for effective control through antibiosis is in place. Expertise in phage biology and biocontrol is sporadic among researchers studying SRE–plant interactions, so both funding and training will likely be required to further develop these control methods.

2.3.4 Inhibitors of SRE Pathogenicity or Metabolism Genes

Interference with bacterial quorum sensing provided promising results for control of soft rot in potato, but this solution is not commercially viable for a number of reasons, one of which is

the expense of development and lack of acceptance for transgenic vegetable crops. Oddly enough, consumers find it acceptable for vegetable crops to be sprayed with a range of synthetic chemicals, including endocrine inhibitors and obesogens in order to control pests and manage diseases. In general, though, crops are not sprayed with antibiotics, which can effectively control bacterial diseases, at least in the short term, because these chemicals are reserved for treatment of disease of humans and other animals.

Researchers are now searching for chemicals that are not antibiotics, but rather that inhibit specific virulence systems (Li et al. 2009). For the most part, plant and animal pathogens use similar virulence and regulatory systems for pathogenicity, so even if useful chemicals are discovered, they will likely be reserved for treatment of animal pathogens. One of the few types of virulence genes unique to the SRE and other similar bacterial decay pathogens, like the pectolytic pseudomonads, are the PCWDE. As yet, no chemical inhibitor of PCWDE has been discovered.

2.3.5 From Genomics to Sustainable Plant Production

By obtaining and analyzing SRE genome sequences, we now know far more about the genetics, evolution, and molecular virulence strategies of SRE pathogenicity than we did a decade ago, but we still have much work to do to put our knowledge into practice in order to improve sustainability of food, fiber, fuel, and ornamental plant production. If we are willing to invest in the necessary research and training, a combination of plant and microbe genomics has the potential to transform plant production in the near future. Control of SRE soft rot and stem rot in potato is an excellent example of this. Potato farmers of the future could feasibly be planting their fields only with hybrid diploid tubers grown from true seed that was planted into hydroponic systems rather than with tetraploid potato that has been through several generations

of field production. The simplification of the seed system would reduce land, water, and fuel use, would reduce the risk of losses due to inclement weather, would simplify planning, and would eliminate the many SRE disease problems that occur in the field and in storage. In addition, these diploid potatoes, for which the entire genome sequence could be known, could carry resistance genes to SRE and many other pests and pathogens that were derived from wild relatives. Genome sequences of these wild relatives would aid in marker-assisted selection of breeding lines encoding these resistance genes. If a new SRE strain emerges, as recently happened with *D. solani* in Europe, its genome could be sequenced and detection tools could be developed and tested within a few weeks. Farmers could use these detection tools to detect SRE strains within their seed supply, soil, and water to enable them to improve their planting and storage decisions. Genome sequences could also be used to make decisions about control options, such as whether a commercially available phage could be used to help control the SRE pathogen in water, soil, or on plants.

Together, these genomics-enabled decisions on variety development, pathogen detection, and pathogen control have the potential to increase efficiency and reduce year-to-year variability, which would simplify farm management and food systems planning. But, little of this is likely to happen if our society does not invest in research and training in both fundamental and transformational plant and microbial sciences.

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