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Mini review

Characterization of pectolytic erwinias as highly sophisticated pathogens of plants*

Solke H. De Boer
Centre for Animal and Plant Health, Canadian Food Inspection Agency, 93 Mount Edward Road, Charlottetown, PEI, C1A 5T1, Canada (Fax: 1-902-368-0960; E-mail: deboers@inspection.gc.ca)

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Abstract

Erwinia carotovora and *Erwinia chrysanthemi* are the two most important soft rotting bacteria of commercially-grown plants. They are genetically diverse as is evident from polymorphisms in the *pel* and *recA* genes as well as in *rrn*, the ribsomal gene cluster. Subpopulations grouped into biovars, pathovars, or subspecies associated with various hosts and in different geographic regions suggest specialization in host preference and/or survival in diverse environments. Previous characterization of the pectolytic erwinias as opportunistic pathogens is being replaced by a realization that this group of bacteria exhibits a sophisticated repertoire of pathogenicity and virulence genes and regulators. The presence of an entire *hrp* gene cluster and associated type III secretion system, and global regulators which regulate virulence determinants such as exoenzyme production and motility, attest to a highly specialized pathogen. The fact that production of extracellular plant cell wall-degrading enzymes are coordinately activated by the diffusible signal molecule *N*-acyl-homoserine lactone in a population density-dependent manner may explain the occurrence of pectolytic erwinia in asymptomatic plant tissues. Transgenic plants expressing bacterial quorum-sensing signal molecules modulate this sensory system and exhibit resistance to soft rot infection. The pectolytic erwinias, being significant plant pathogens that are neither of quarantine concern nor a human health hazard while readily isolated from field sources, make an ideal model for investigating the genetic basis of plant pathogenesis and environmental fitness.

Introduction

The enterobacter-like plant pathogens which macerate and decay plant tissue, often referred to as the pectolytic erwinias, reside in the genus *Erwinia* named after the eminent plant pathologist, Erwin F. Smith. Two species, *Erwinia carotovora* and *Erwinia chrysanthemi*, traditionally circumscribed the important plant pathogenic strains but reclassification into multiple species in a new genus, *Pectobacterium*, has been proposed (Gardan et al., 2003).

Previous suggestions to separate the pectolytic enterobacteria into the genus *Pectobacterium* has not found favor among phytobacteriologists. Initially the suggestion was made by Waldee (1945), who recommended the segregation on the basis of the unique pectolytic activity of the bacteria. Subsequently, Hauben et al. (1998) revived the suggestion and added evidence from sequence analysis of the 16S ribosomal DNA of various plant-associated members of the *Enterobacteriacae* to support the proposal. Although phenotypic characterization and analysis of a single DNA fragment might have been considered insufficient for subdivision at the generic level, the DNA : DNA hybridization study conducted by Gardan et al. (2003) provides further stimulation to change in favor of the new nomenclature. In this review, however, I will remain with the familiar current usage of *Erwinia* as the genus name.

The pectolytic erwinias are ubiquitous in environments that support plant growth, and because they

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may be found in association with asymptomatic plants, they have been viewed as opportunistic pathogens analogous to medical bacteria that infect only immunologically compromised individuals. However, in this brief review I wish to show that recent data supports a different view. The emerging picture is of pectolytic erwinias as a group of diverse ecologically-adapted bacteria that interact with host plant species at a level of sophistication that rivals other genera of phytopathogenic prokaryotes. I will review progress in our understanding of their diversity, ecological fitness, and pathogenicity.

Diversity

For many years it was recognized that *E. carotovora* was comprised of two different strain clusters. One cluster was more or less restricted to strains associated with the blackleg disease of potato and has been known since 1969 as *E. carotovora* subsp. (or var.) *atroseptica* (Dye, 1969). The *atroseptica* strains are differentiated from other *E. carotovora* strains by their inability to grow at 36 °C, production of acid from α-methyl glucoside, and production of reducing substances from sucrose. Strains of *E. carotovora* that do not conform to these criteria were classified as *E. carotovora* subsp. *carotovora*. More recently, other subspecies, namely, *betavasculorum* (Thomson et al., 1981) *wasabiae* (Goto and Matsumoto, 1987), *odorifera* (Gallois et al., 1992), and *brasiliensis* (Duarte et al., 2003) have been delineated on the basis of biochemical and physiological criteria to represent clusters of strains associated with specific plant diseases. Generally, the subspecies taxons are supported by molecular and sequence data. Analysis of restriction fragment length polymorphisms of PCR amplicons generated from the intergenic spacer in the ribsomal (*rrn*) operon and the *recA* and *pel* genes (*rec* for recombination, *pel* for pectate lyase) differentiate the subspecies of *E. carotovora* and distinguish them from *E. chrysanthemi* (Helias et al., 1998; Toth et al., 2001; Waleron et al., 2002). Similarly, amplified fragment length polymorphisms of genomic DNA reveal strain clusters consistent with subspecies identification. Phylogenetic trees constructed on the basis of 16S DNA sequences and on limited sequence data for the intergenic spacer region of the small *rrn* operon, also identified the subspecies as separate taxons (Fessehaie et al., 2002). The recent study by Gardan et al. (2003), in which DNA : DNA hybridization was used for inter-subspecies comparisons, not only supported the subspecies identifications but provided data to promote many of them to species rank.

Erwinia chrysanthemi differs from *E. carotovora* in its organization of genes for pectic enzymes, and in DNA sequences of key regions such as 16S ribosomal genes and IGS regions. In practice, *E. chrysanthemi* has been differentiated from the other pectolytic erwinia on the basis of indole production, phosphatase activity, and erythromycin sensitivity. Diversity within the species is related to host specificity and is captured by division of the species into biovars or pathovars. Recently it has been proposed, on the basis of quantitative hybridization and phenotypic characterization, that subgroups of *E. chrysanthemi* be given species status. Hence the species *Erwinia* (or *Pectobacterium*) *dianthicola, dieffenbachiae*, *chrysanthemi*, *zeae*, *dadanti*, and *E.* (or *Brennaria*) *paradisiaca* were recommended (Samson et al., 2001).

Genetic diversity at the subspecific level is recognized in *E. carotovora* serotypes based on differences in the lipopolysaccharide O antigen (De Boer et al., 1979) and in differences in the O and H antigens of *E. chrysanthemi* (Janse and Ruissen, 1988). Additional subspecific diversity is evident in discriminatory phage sensitivity (Toth et al., 1999), and banding patterns achieved by PCR–RFLP protocols (Boccara et al., 1991; Nassar et al., 1996; Waleron et al., 2002). There are also significant phenotypic differences among strains within subspecies.

Ecological fitness

It is one thing for a single-bacterial species to be ubiquitously present in many diverse environments, but quite another thing for different ecologically-adapted strains to occupy specific habitats suited to their unique characteristics. While the first perception, in particular, emerges from data relating the presence of *E. carotovora* subsp. *carotovora* to many different hosts and in many different milieus including insects, soil, snow, clouds, fresh surface water, and coastal salt water, these studies generally have failed to consider differences among the isolated strains. The plausible scenario of pectolytic erwinias off the Pacific coast, aerosolized by wave activity, being carried at high altitudes over the Rocky Mountains and being deposited in irrigation water and on potato fields in Colorado by rain showers, needs to be confirmed by monitoring the characteristics of strains found at each location to be sure that different populations of the bacteria do not reside

at the different sampling sites (Harrison et al., 1987; Maddox and Harrison, 1988). Earlier work on potato indicated that strains isolated from foliage (predominantly serogroup XVIII) in the Pemberton Valley of British Columbia were a different serotype than those found on belowground portions of the plant (predominantly serogroup III) (De Boer, 1983). The obvious assumption that tubers from *Erwinia*-free stem cuttings would become contaminated with *Erwinia* washed by rain from aerial sources deposited on leaf surfaces was not substantiated in this study. Similarly, when *Erwinia*-free potato plants were artificially contaminated with a foreign potato isolate of *E. carotovora* subsp. *carotovora* (serotype X) and having survived for a full growing season, was found to have been replaced the following spring on volunteer plants by serotype III strains that commonly occur on potato in that geographic area (De Boer, 1979). Specific ecologically-adapted strains tend to survive and predominate in those environments to which they are best adapted to the exclusion of other strains.

The discovery that different subspecies are associated with specific hosts also supports the notion that *E. carotovora* and *E. chrysanthemi* are not homogeneous species involved in pathogenesis of many plant species. Rather specific, identifiable subgroups of pectolytic erwinia are found in specific ecological niches. Hence *E. carotovora* subsp. *betavasculorum* is associated with sugar beet, *E. carotovora* subsp. *wasabiae* with horseradish and *E. carotovora* subsp. *odorifera* with witloof chicory and allium crops. Whether the association of *E. carotovora* subsp. *brasiliensis* with blackleg of potato in Brazil instead of *E. carotovora* subsp. *atroseptica* as found in North America and Europe is due to differences in habitat or due to chance selection of strains in a different geographic area is yet to be determined (Duarte et al., 2003). It is also not yet known to what extent subtypes within subspecies are specific for unique ecological niches. One study did suggest that the genetic diversity of *E. carotovora* subsp. *carotovora* in Japan appears to be related to their host plants (Seo et al., 2000).

The genetic basis of ecological fitness factors are virtually unknown but are expected to be varied and diverse. One ecological fitness candidate is the putative ABC transporter gene, *ybiT*, described in *E. chyrsanthemi*, which gives a competitive advantage against endophytic bacteria *in planta* (Llama-Palacios et al., 2002). Resistance of erwinia strains to inhibitors produced by competing heterologous bacteria, as exemplified by production of 2,4-diacetlyphloroglucinol by *Pseudomonas fluorescens* (Cronin et al., 1997), are important factors not yet characterized. The identification of chrysobactin in *E. carotovora* subsp. *carotovora* in addition to two other catechol siderophores (Barnes and Ishimaru, 1999) suggests that iron sequestration is also a complex fitness factor that may define a suitable niche (Expert, 1999).

Pathogenicity

Exoenzymes are the primary virulence factors of pectolytic erwinias. The exoenzymes pectate lyase, pectin lyase, and polygalaturonase, are directly involved in plant tissue maceration by digesting the pectin that cement plant cells together. Others such as proteases and cellulases contribute to virulence by augmenting the activity of the pectic enzymes. However, there is more to pathogenicity than maceration alone. Purified pectic enzymes applied to plant tissue can cause maceration, and simple transformation of *Escherichia coli* with the genes for pectate lyase does not transform *E. coli* into a plant pathogen even though such transformed bacteria can incite tissue maceration (Keen and Tamaki, 1986; Lei et al., 1985). Pathogenicity also involves invasion of plant tissue and eluding plant defense mechanisms.

Although we are far from having a complete picture of pathogenicity for any plant pathogenic bacteria, it is becoming crystal clear that virulence factors are under tight genetic control. The genetic control apparatus in pectolytic erwinias is no less complex than in *Ralstonia solanacearum* (Schell, 2000) or *Pseudomonas syringae* (Hirano and Upper, 2000). The sophisticated regulatory mechanisms may be masked when erwinias cause decay in metabolically dormant or excised plant parts; they are nevertheless essential for causing disease in living host plants. Exoenzymes are under strict transcriptional and postranscriptional control by regulatory mechanisms which includes cell density dependent quorum sensing and response to plant signals.

Global regulatory genes such as *hexA* and *hexY* (*hex* for hyperproduction of exoenzymes) probably repress a cascade of secondary regulators since mutations in these genes leads to overproduction of extracellular enzymes and concomitant hypermotility and production of harpin, the protein involved in eliciting the hypersensitive response (Harris et al., 1998; Shih et al., 1999). The review by Hugouvieux-Cotte-Pattat et al. (1996) on the pectinolysis in *E. chrysanthemi* and the

work of A.K. Chatterjee's laboratory and others on *E. carotovora* show that the pectolytic erwinias possess various genes of a regulatory cascade that are analogous to genes associated with pathogenicity in medically important enterics (Mukherjee et al., 2000). The *kdgR* gene (*kdg* for 2-keto-3-deoxygluconate general repressor) product, for example, which negatively regulates the genes involved in pectin degradation by binding to the operator regions, has a high degree of similarity to the deduced amino acid sequence of KdgR in *E. coli* (Liu et al., 1999; Reverchon et al., 1991). Similarly the *rsmA* and *rsmB* (*rsm* for repressor of secondary metabolites) genes which negatively regulate exoenzyme production is found in many pectolytic bacteria and are homologs of *csrA* and *csrB* (*csr* for carbon storage regulator), respectively, in *E. coli* where they control glycogen accumulation, cell size, and cell surface properties (Cui et al., 1995). In fact, the gene pair modulate the expression of many genes in a unique fashion. RsmA, as a RNA-binding protein, promotes message decay and is uniquely neutralized when bound by *rsmB* RNA in an inactive nucleoprotein complex (Liu et al., 1998). Production of both RsmA and *rsmB* RNA are regulated by a third component, *rsmC* of this global regulatory system (Cui et al., 1999). Mukherjee et al. (2000) speculate on how *hexA*, *kdgR*, the RpoS alternate sigma factor, and the *rsm* regulators interact to govern virulence factors in *E. carotovora* subsp. *carotovora*. Even more rigorous control over exoenzyme and harpin production appears to be achieved by involvement of the two-component *gacA/gacS* system (*gac* for global activator sensor kinase) which regulates these virulence factors in a positive way (Eriksson et al., 1998) and may do so by regulating *rsmB* transcription (Cui et al., 2001).

Bacterial pathogenicity requires mechanisms to transport weaponry for assault on host components to the outside of the cell in which they are produced. To this end, plant pathogenic bacteria have discreet secretion systems. In pectolytic erwinias, proteases are secreted directly through the cell envelop without periplasmic intermediates via the type I pathway. However, the pectinases and cellulases utilize a type II secretory system, a two-step process which includes an intermediary periplasmic stage. The type II apparatus of *E. carotovora* subsp. *carotovora* is encoded by some 15 genes in the *out* cluster (Thomas et al., 1997). The importance of a third secretory system (type III) for pathogenicity is deduced from the presence of the *hrp* gene cluster (*hrp* for hypersensitivity reaction and pathogenicity) in *E. chrysanthemi* and *E. carotovora* (Bell et al., 2002; Rantakari et al., 2001; Yang et al., 2002). *hrp* genes designate the type III secretion pathway in other plant pathogenic bacteria and are essential for virulence (Hueck, 1998). Although mutations in *hrp* genes of *E. chrysanthemi* did not greatly affect its ability to macerate plant tissue in experimental inoculation studies, it may serve a crucial role in pathogenesis in natural settings (Yang et al., 2002). Since the type III system serves to transfer effector proteins via a pilius-dependent mechanism into host cells in bacterial/animal pathosystems (Hueck, 1998), it is tempting to speculate that pectolytic erwinia also communicate with the host plant via the *hrp* system. Both harpin, the apparent effector protein which causes the hypersensitive response, and genes of the type III pathway are under tight regulatory control (Chatterjee et al., 2002). Control mechanisms of many genes within the *hrp* cluster are yet to be elucidated as is a clear role for the type III secretory system. Perhaps it is this portion of the pathogenicity apparatus that determines host specificity.

The discovery of density-dependant quorum sensing has added a whole new dimension to our understanding of how bacteria respond and interact with their environment. Like many gram negative bacteria, the erwinias utilize an acyl-homoserine lactone (AHL) as a signal molecule and it regulates expression of pectic exoenzymes. Whether AHL production is regulated by the Rsm system (Mukherjee et al., 2000) or is itself a regulator of *rsmB* (Koiv and Mae, 2001), it is clear that modulation of AHL affects pathogenicity. Experimental tobacco and potato plants genetically modified to produce *N*-acyl-homoserine lactonase were significantly more resistant to *E. carotovora* than non-transformed plants (Dong et al., 2001), presumably because the hydrolyzing enzyme inactivated the microbial AHL. If indeed the exoenzymes required for disease initiation are activated only when population density surpasses a set threshold, the pectolytic bacteria may reside quite unobtrusively at low densities inside plants. Their presence among endophytic populations of ostensibly healthy plants has been observed (Helias et al., 2000; Surette et al., 2003). My own research showed that *E. carotovora* subsp. *atroseptica* can be present in potato stems, stolons, and tubers without manifestation of disease (unpublished), as well as in micropropagated plantlets growing on artificial media (Lan, 1991). The widespread presence of *E. carotovora* subsp. *atroseptica* at the stolon attachment site of

asymptomatic tubers (De Boer, 2002) may represent one aspect of the bacterium's strategy for self preservation and dissemination as innocuous microflora, only to blossom as a decay-inducing organism when the population density increases, AHL accumulates, and exoenzymes macerate plant tissue to provide a large nutrient sink.

Conclusion

Despite the negative aspects of the enormous loss in agricultural crops caused by bacterial decay, one can only appreciate the complexity of the inciting bacteria at both the population and molecular levels. The current state of knowledge allows us to view pectolytic bacteria as much more than just producers of macerating enzymes. Specialized members within a complex population structure thrive in specific niches wherein they exist as benign members of the microbial flora and yet possess the apparatus of a devastating plant pathogen held in check by an intricate and delicately balanced regulatory system. Expression of virulence and resulting plant destruction, from the bacterium's perspective, is a clever means of accessing a large nutrient source.

As knowledge of bacterial genetics is augmented by sequencing initiatives and the teasing out of regulatory pathways, it will be possible to come to a deeper understanding of what is involved in bacterial survival and adaptation. In many ways, the pectolytic erwinias are an ideal model system to pursue these studies since this group of bacteria is phylogenetically diverse, is easily isolated, is not usually of medical or quarantine concern, is an economically important bacterium, and is amendable to laboratory manipulation. Whatever the outcome, the pectolytic erwinias have earned a respectable and even noteworthy place among the other phytopathogenic prokaryotes.

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