Research Note

Identification of a Pathogenicity Locus, *rpfA*, in *Erwinia carotovora* subsp. *carotovora* That Encodes a Two-Component Sensor-Regulator Protein

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A mutant of *Erwinia carotovora* subsp. *carotovora*, AH2552, created by a *Mud1* insertion was found to be reduced in plant pathogenicity and deficient in extracellular protease and cellulase activity, although it produced normal levels of pectate lyase and polygalacturonase. A cosmid clone, pEC462, was isolated from a wild-type *E. carotovora* subsp. *carotovora* DNA library that concomitantly restored pathogenicity and protease and cellulase activities of AH2552 to wild-type levels when present in trans. The genetic locus that was disrupted in AH2552 by insertion of *Mud1* has been designated *rpfA*, for regulator of pathogenicity factors. Sequencing of the *rpfA* region identified an open reading frame of 2,787 bp, and the predicted 929-amino acid polypeptide shared high identity with several two-component sensor-regulator proteins: BarA from *Escherichia coli*, ApdA from *Pseudomonas fluorescens*, PheN from *P. tolaasii*, RepA from *P. viridiflava*, LemA from *P. syringae* pv. *syringae*, and RpfC from *Xanthomonas campestris* pv. *campestris*. The *RpfA* locus described in this study encodes a putative sensor kinase protein that is involved in both extracellular protease and cellulase production and the pathogenicity of *E. carotovora* subsp. *carotovora* on potato tubers.

Additional keywords: histidine protein kinase, pectolytic enzymes.

Erwinia carotovora subsp. *carotovora* is a member of the "soft-rot" group of erwinias and is capable of causing tissue maceration and disease among a broad host range of plants, especially potato tubers held in storage (Perombelon 1987; Perombelon and Kelman 1980). The production of extracellu-

lar enzymes such as cellulase (Cel), protease (Prt), pectate lyase (Pel), and polygalacturonase (Peh), which depolymerize plant cell walls, has long been correlated with the virulence of *E. carotovora* subsp. *carotovora* strains (Chatterjee and Vidaver 1986; Collmer and Keen 1986; Kotoujansky 1987). Further evidence that pectolytic enzymes play a role in the pathogenicity of *E. carotovora* subsp. *carotovora* and the related soft-rot erwinias, *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*, is that avirulent mutants have been isolated that are either deficient or defective in the production or secretion of these enzymes (Boccaro et al. 1988; Hinton et al. 1989; Pirhonen et al. 1991).

Random insertional mutagenesis and recombinant DNA strategies have resulted in the identification and subsequent cloning of several regulatory genes that control extracellular enzyme production and virulence in *E. carotovora* subsp. *carotovora*. The genes *aepA* and *aepH* have been found to be activators of extracellular protein production. *aepA* has been shown to activate transcription of *pel-1*, the major Pel isozyme in *E. carotovora* subsp. *carotovora* (Murata et al. 1991; Liu et al. 1993), and multiple copies of *aepH* activate the production of Pel, Peh, Cel, and Prt in *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *Escherichia coli* (Murata et al. 1994). The gene *carI/expI* is related both structurally and functionally to the *luxI* gene product of *Photobacterium fischeri* in that it directs the production of an auto-inducer molecule identical to the *luxI* synthesized autoinducer *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL) (Jones et al. 1993; Pirhonen et al. 1993; Salmond et al. 1995). OHHL regulates the production of Pel, Cel, Peh, and Prt and the synthesis of the antibiotic carbapenem in *E. carotovora* subsp. *carotovora* (Jones et al. 1993; Pirhonen et al. 1993). A LuxR homologue, encoded by *rexR/expR*, has been found directly downstream of *carI/expI*; however, its role as a transcriptional regulator has not been demonstrated (Salmond et al. 1995). A negative regulator gene, *rsmA*, has been identified that suppresses the production of Pel, Peh, Cel, and Prt; suppresses OHHL synthesis; and reduces plant pathogenicity in several soft-rotting *Erwinia* spp. (Cui et al. 1995; Chatterjee et al. 1995).

Previously, sixty-three mutants of *E. carotovora* subsp.

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The GenBank accession number for the DNA sequence of the *rpfA* gene of *Erwinia carotovora* subsp. *carotovora* AH2 is U62023.

carotovora impaired in pathogenicity (Path⁻) were generated by insertion of *Mud1* (Handa et al. 1987a, 1987b). Among the 63 Path⁻ mutants that were isolated, 46 were prototrophic, 16 were defective in the synthesis of purines and pyrimidines, and one was impaired in galactose utilization (Jayaswal et al. 1985). Most of the prototrophic mutants (42 of 46) were defective in the secretion of either Peh, Pel, or both (Handa et al. 1987b). However, four independent mutants, AH2024, AH2552, AH2819, and AH4334, were not impaired in the synthesis or secretion of Pel or Peh (Handa et al. 1987b). Growth rates of these mutants in minimal salts medium containing glucose and isoelectric profiles of secreted Pel isoforms were identical to those of the wild-type strain AH2 (data not shown). However, further characterization of AH2552 in this study revealed it was defective for the pro-

duction of extracellular protease and cellulase activities (Fig. 1).

We cloned and characterized the genetic locus disrupted by the *Mud1* insertion in the nonpathogenic mutant, AH2552, in order to decipher its pleiotropic nature. We hereby designate this locus *rpfA* for regulator of pathogenicity factors. A genomic library of AH2552 (in EMBL3) was screened by hybridization with the *Mud1* fragment from pJC101 (Table 1), and several clones were isolated. After restriction sites within the inserts of the clones were mapped, a common 5.0-kb *EcoR1-HindIII* junction fragment containing *Mud1* was identified. Subsequently, this 5.0-kb *EcoR1-HindIII* *Mud1::AH2552* junction fragment was used as a hybridization probe to screen a genomic DNA library from wild-type strain AH2. The AH2 library was constructed in the *Bam*H1 site of cosmid vector pLAFR3 after partial digestion with *Sau*3A and size fractionation on a 10 to 40% sucrose gradient.

Nine cosmid clones were isolated from the *E. carotovora* subsp. *carotovora* AH2 genomic library (approximately 1,000 clones) that hybridized with the *Mud1::AH2552* probe and were mobilized from HB101 via triparental matings with the helper plasmid pRK2013 (Ditta et al. 1980) into *E. carotovora* subsp. *carotovora* mutant AH2552. Transconjugants from each of the matings were tested for their ability to restore wild-type phenotypes to strain AH2552. Cosmid clone pEC462 restored extracellular protease and cellulase activities (Fig. 1) and the ability to macerate potato tuber tissue (Table 2). Plate assays and isoelectric focusing gels of *E. coli* strains containing pEC462 did not show cellulase or protease activity, indicating that pEC462 did not contain *Prt* (Kyostio et al. 1991; Tang et al. 1987) or *Cel* (Saarilahti et al. 1990; Cooper and Salmond 1993) genes (data not shown).

Fig. 1. Plate assays for the production of the extracellular enzymes **A**, protease; **B**, cellulase; and **C**, pectate lyase by *Erwinia carotovora* subsp. *carotovora* strains AH2, AH2552, and AH2552 containing pEC462. Enzyme activity was determined for *E. carotovora* subsp. *carotovora* strains by spotting 5 μ l from cultures (5×10^8 cfu/ml of Luria broth grown overnight at 28°C) using standard plate assays for protease (Tang et al. 1987), pectate lyase (Keen et al. 1984), and cellulase (Andro et al. 1984).

Table 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics ^a	Reference
Strain		
<i>Escherichia coli</i>		
HB101	<i>thr leu thi recA hsdR hsdM pro</i> Sm ^r	Boyer et al. 1969
DH5 α	F ⁻ <i>recA lacZ</i> Δ M15	BRL ^b
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>		
AH2	Str ^r Nal ^r pathogenic pectolytic	Jayaswal et al. 1984
AH2552	AH2::Mud1(Ap ^r <i>lac</i> <i>cts</i> 62)	Handa et al. 1987a
Plasmid or vector		
EMBL3	λ sbh1 λ 1 ^b 189 (polycoding site <i>int</i> 29 <i>nin</i> L44 <i>trpE</i> polycoding site)	Frischauf et al. 1983
	KH54 <i>chiC</i> <i>srI</i> 4 ^o <i>nin</i> 5 <i>srI</i> 5 ^o	
pLAFR3	IncP Tc ^r Cos ⁺ <i>rlx</i> ⁺ ; cloning vector	Staskawicz et al. 1987
pBluescript SK ⁺	Ap ^r ; cloning vector	Stratagene ^c
pUC19	Ap ^r ; cloning vector	BRL ^b
pRK2013	IncP Km ^r Tra RK2 ⁺ ; helper plasmid for triparental matings	Ditta et al. 1980
pPO1681	21.4-kb <i>Bam</i> H1 internal deletion of <i>Mu</i> II 7701-301 from pPO1669	Castillo et al. 1984
pJC101	3.0-kb <i>Eco</i> R1- <i>Bam</i> H1 fragment containing the right end of <i>Mud1</i> from pPO1681 cloned into pUC19	This study
pEC462	21.0-kb <i>rpfA</i> ⁺ clone in pLAFR3 from <i>E. carotovora</i> subsp. <i>carotovora</i>	Handa et al. 1987a
pPS3.1	5.0-kb <i>Pst</i> I- <i>Sal</i> I fragment from pEC462 cloned in pBluescript SK ⁺	This study
pHS1.2	3.8-kb <i>Hind</i> III- <i>Sal</i> I fragment from pEC462 cloned in pBluescript SK ⁺	This study
pES6.1	0.9-kb <i>Sal</i> I- <i>Eco</i> R1 fragment from pEC462 cloned in pBluescript SK ⁺	This study
pEK5.1	2.3-kb <i>Kpn</i> I- <i>Eco</i> R1 fragment from pEC462 cloned in pBluescript SK ⁺	This study
pEB4.1	4.6-kb <i>Bam</i> H1- <i>Eco</i> R1 fragment from pEC462 cloned in pBluescript SK ⁺	This study

^a Ap^r, Nal^r, Rif^r, Tc^r, Sm^r, and Sp^r = resistant to ampicillin, nalidixic acid, rifampicin, tetracycline, streptomycin, and spectinomycin, respectively.

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Previously, we reported (Handa et al. 1987b) that the *MudI* insertion in AH2552 was located within a 6.7-kb *EcoR*I DNA fragment. In order to map more precisely the location of the *MudI* insertion in AH2552, the 6.7-kb *EcoR*I fragment from pEC462 was used as a hybridization probe against Southern blots of genomic DNA of AH2552 digested with various

Table 2. Wet weight (g) of potato tuber tissue macerated by *Erwinia carotovora* subsp. *carotovora* strains AH2, AH2552, and AH2552 (pEC462)^a

Strain	Inoculation method	
	Stab	Overlay
AH2	0.143 ± 0.020	0.165 ± 0.013
AH2552	0.012 ± 0.005	0.011 ± 0.005
AH2552 (pEC462)	0.164 ± 0.008	0.157 ± 0.013

^a Surface-sterilized potato tubers were cut with a no. 6 cork borer (1.2 cm in diameter) and sliced into disks approximately 5 to 10 mm thick. Pathogenicity tests were conducted by inoculating each disk with 5 µl from a culture (5×10^8 cfu/ml of Luria broth medium grown overnight) of the appropriate *E. carotovora* subsp. *carotovora* strain. Two sets of inoculations were done. In one, the bacteria were placed directly on top of the potato tuber slices (overlay); in the other, the inoculum was stabbed into each potato slice with a micropipette tip. Each strain tested was inoculated onto four potato slices for both types of inoculation assay. The potato disks were placed on top of moist Whatman filter paper in petri plates, which were then covered and sealed with paraffin. After the plates were incubated at 28°C for 48 h, the rotted tissue was scraped from each potato disk and weighed.

restriction enzymes and compared with the same genomic DNA digests of AH2 (data not shown). The *MudI* insertion mapped approximately 0.4 kb to the left of the *SalI* site within the 6.7-kb *EcoR*I fragment contained within the pEC462 insert (Fig. 2B). Subclones derived from the 6.7-kb *EcoR*I fragment of pEC462 were electroporated into AH2552 and assayed for their ability to restore pathogenicity when inoculated onto potato tuber slices. None of the subclones shown in Figure 2C were able to restore pathogenicity or extracellular protease and cellulase activity to AH2552, therefore suggesting that the *rpfA* locus extended to the right of the *EcoR*I site shown in Figure 2B.

The nucleotide sequence of the 6.7-kb *EcoR*I fragment revealed an incomplete open reading frame (ORF) spanning the site of the *MudI* insertion in AH2552 that continued to the right of the *EcoR*I site (Fig. 2B). A complete ORF consisting of 2,787 bp, which spanned the *EcoR*I site and encoded a protein of 929 amino acids, was identified after nucleotide sequencing of the adjacent 4.0-kb *EcoR*I fragment (GenBank accession number U62023). Analysis of the nucleotide sequence downstream of this ORF did not reveal any other ORFs in the same orientation that could have been disrupted as the result of polar effects of the *MudI* insertion (data not shown). Upstream of the predicted ATG translational start codon, there is a putative Shine-Dalgarno sequence (GGAA) beginning at position 122. The nucleotide sequence upstream of the ATG codon was analyzed for promoter sequences, but

Fig. 2. A, Restriction map of the pEC462 insert cloned in pLAFR3. Restriction sites: B, *Bam*H1; P, *Pst*I; S, *Sal*I; K, *Kpn*I; E, *Eco*R1; H, *Hind*III; and N, *Nco*I. B, Location of the *MudI* insertion site and the *rpfA* locus. The arrow denotes the direction of transcription. C, Fragments of the *rpfA* region subcloned in pBluescriptSK used in complementation analysis and DNA sequencing.

neither -35/-10 consensus promoter sequences nor σ^{54} -type consensus promoter sequences were found. Two long, uninterrupted stretches of hydrophobic amino acids occur in the N-terminal portion of RpfA at residues 10 to 31 and at residues 176 to 198, which could function as membrane-spanning regions (Kyte and Doolittle 1982). Such motifs are common to response regulators and to some sensor kinases of two-component regulatory proteins (Parkinson and Kofoed 1992; Stock et al. 1995).

When the deduced RpfA amino acid sequence was compared with other proteins in the current databases, RpfA was found to share significant similarity to members of a class of environmentally responsive prokaryotic proteins known as two-component regulators. To date, the majority of two-component regulatory protein systems are comprised of two different proteins: a histidine protein kinase sensor protein and a response regulator protein that subsequently becomes phosphorylated by the sensor protein (Parkinson and Kofoed 1992; Stock et al. 1995). A subset of the two-component regulatory systems, in which a single protein contains both histidine kinase sensor and response regulator domains, exists in some bacteria. RpfA is a member of this class of proteins, and a comparison of RpfA with the six most similar proteins is shown in Figure 3. Specifically, the deduced amino acid of RpfA shares 60.5% identity and 74.5% similarity with BarA from *E. coli* (Nagasawa et al. 1992), 38.4% identity and 56.5% similarity with RepA from *Pseudomonas viridiflava* (Liao et al. 1994), 38.1% identity and 58.7% similarity with ApdA from *P. fluorescens* (Corbell and Loper 1995), 38.1% identity and 56.8% similarity with PheN from *P. tolaasii* (Grewal et al. 1994, 1995), 37.7% identity and 55.3% similarity with LemA from *P. syringae* pv. *syringae* (Hrabak and Willis 1992), and 29.4% identity and 55.7% similarity with RpfC from *Xanthomonas campestris* pv. *campestris* (Tang et al. 1991). All of the individual residues that are conserved in most histidine kinase sensor and response regulator pairs are present in the deduced RpfA amino acid sequence. ApdA, which regulates an array of secondary metabolites in *P. fluorescens* including antibiotic production, was shown to cross-complement a *P. syringae* pv. *syringae* LemA⁻ mutant for extracellular protease production (Corbell and Loper 1995). The precise physiological role of BarA is unclear at the present time, although it has been shown to suppress an *envZ* mutation in *E. coli* (Nagasawa et al. 1992).

The sensor kinase proteins in two-component systems are believed to undergo autophosphorylation in response to a signal molecule or molecules produced after changes in environmental conditions (Parkinson and Kofoed 1992). The transmembrane sensor proteins contain an ATP-binding site and a conserved histidine residue that becomes the site of autophosphorylation upon activation (Parkinson and Kofoed 1992). Whether the sensor protein acts directly as a receptor for the environmental signal or in an intermediate role in the signal transduction is still unclear in most cases. The phosphorylated form of the sensor protein then acts as a kinase and transfers the phosphoryl group to a conserved aspartic acid residue in the receiver domain of the cytoplasmic response regulator protein (Parkinson and Kofoed 1992). Upon phosphorylation, the response regulator activates transcription of a set of genes that brings about the observed phenotypic changes to the altered environmental conditions (Parkinson and Kofoed 1992;

Stock et al. 1995). Analysis of the RpfA sequence shows that the conserved histidine and aspartic acid residues are present at position 301 in the putative sensor domain and at position 725 in the putative response regulator domain, respectively. However, RpfA, like BarA, RepA, ApdA, PheN, LemA, and RpfC, lacks a helix-turn-helix motif in the putative response regulator region that would make direct recognition of target promoter regions unlikely (Brennan and Matthews 1989).

Recently, *gacA* in *P. syringae* pv. *syringae* was shown via genetic analysis to encode the response regulator for LemA (Rich et al. 1994). The *gacA* gene was first described in *P. fluorescens* as a regulator of secondary metabolites that are involved in fungal suppression (Laville et al. 1992). The *gacA* gene encodes a protein of 221 amino acids that contains the conserved residues characteristic of response regulators and a helix-turn-helix domain in the carboxy-terminal region of the protein, which could serve to activate transcription of target promoters (Rich et al. 1994). Whether the *repB* locus that has been identified close to the *repA* gene in *P. viridiflava* turns out to be a *gacA* homologue remains to be determined. It is tempting to speculate that because of the high degree of similarity between RpfA and LemA, there might be a corresponding *gacA* homologue in *E. carotovora* subsp. *carotovora* that would act as a transcriptional regulator upon activation by a phosphorylated version of RpfA.

Despite the high degree of similarity between RpfA and RepA from *P. viridiflava* and RpfC from *X. campestris* pv. *campestris*, Liao et al. (1994) were unable to detect any homologues of *repA* by Southern blot analysis of digested genomic DNA from various strains of *Erwinia* and *Xanthomonas* spp. A comparison of the nucleotide sequences for the coding regions of the *RpfA* and *RepA* genes shows that they share only 50.2% identity at the nucleotide level, which would have been below the level of detection for standard Southern blot hybridizations. The other important point concerning RepA and RpfA is that a *P. viridiflava* RepA mutant is also defective in the production of pectate lyase, whereas a *rpfA* mutation does not affect pectate lyase production in *E. carotovora* subsp. *carotovora*.

Prokaryotes have been found to respond to various kinds of alterations in their environment by using a conserved signal transduction mechanism that activates specific sets of genes that allow the bacterium to deal with new environmental conditions (Parkinson and Kofoed 1992; Stock et al. 1995). A signal transduction mechanism for this adaptation is the two-component histidine kinase sensor and response regulator system (Parkinson and Kofoed 1992; Stock et al. 1995). The regulation of pathogenicity factors in *E. carotovora* subsp. *carotovora*, a soft-rot phytopathogen, is a complex system that involves several different levels of regulation for the synthesis and export of extracellular proteins. Already, the genes *aepA*, *aepH*, *carI/expI*, and *rsmA* have been shown to regulate the production of extracellular proteins in *E. carotovora* subsp. *carotovora* (Murata et al. 1991; Liu et al. 1993; Pirhonen et al. 1991, 1993). With the discovery of *rpfA*, we now show that there is an additional level of regulation to the production of extracellular protease and cellulase and pathogenicity in *E. carotovora* subsp. *carotovora*. Since most of the prokaryotic two-component response regulators act as transcriptional activator proteins, it follows that RpfA might act as a transcriptional activator for *cel* and *prt* gene expression or for addi-

RpfA	MTKYSRLRAR	MLLILLAPTL	MIGLLLSFF	VIHRYNQLOS	QLADSGASTI	49
BarA	MTNYSRLRAR	MMILILAPTV	LIGLLLSIFF	VVHRYNDLOR	QLEDAGASTI	49
RepA		VLLLTILPAS	LMAAVLGGYF	TWMQLYELOS	QLLQRGEMIA	40
ApdA	MLKKLGIKGR	VLLLTLLPTS	LMALVLGGYF	TWMQLSDLOT	QLLQRGEMIA	50
Phen	MLRRMGIKGR	VLLLTLLPTT	LMALLLGGYF	TWMQLSELOT	QLLQRGEMIA	50
LemA		VLLLTILPAS	LMAAMLGGYF	TWMQLSELOS	QLLQRGEMIA	40
RpfC						
RpfA	DAAGNHQRYA	ITHRQMDTIP	ALINTLHRRH	SAIIRITISVF	DARNQLLATI	99
BarA	EPLAVSTEYG	MSLQNRISIG	QLISVLHRRH	SDIVRAISVY	DENNRFLVTS	99
RepA	QDLAPLSANA	LGRKDKILLT	RIAT..QTLE	QSDVRAVSFL	DTDRSVLA..	86
ApdA	EQLASLVAPA	MGNHNTQMLE	RIAT..QALE	QSDVRAVSLL	APDRSLLA..	96
Phen	EQLAPLVAPA	LSTRNTDLE	RIAT..QSLE	QPDVRAVSFL	APDRSPLA..	96
LemA	QDLAPLAANA	LGRKDKVLLS	RIAT..QTLE	QTDVRAVSFL	DTDRTVLA..	86
RpfC						
RpfA	KLRNKRTLQC	LSNDVLAHNK	PHLHHTNDAL	ILHMTIVTDS	EFMAGTTPVL	149
BarA	NFHLDPSSMQ	LGSNVFPFRQ	LTVTRDGDIM	ILRTPIISES	.YSPDESPSS	148
RepA	..HAGPTMIS	.PSPIGSSQ	PLNSSGNDAT	RYLLPVFGHQ	RHLTSP...I	130
ApdA	..HAGPSMLN	.PPPAGNSSH	MMQRSGSDAT	RYQLPVFGRH	RNLAGE...L	140
Phen	..HARPTMLN	.QPPVGNSSH	FVAASGNDAT	RYLLPVFGRH	RNLAGE...L	140
LemA	..HAGPTMIS	.PSPIGSSQ	PLSSTGTAT	RYLLPVFGSQ	RHLTSP...I	130
RpfC		MATWLILVGE	PLVSLGLMVA	ILLRPQVSHT	RRLIG.....	35
RpfA	PGTATP..LG	YLVIELDTST	IRLQQYQEIF	IAALLLLSL	GAAVIFAYEL	197
BarA	DAKNSQNMIG	YIALELDLKS	VRQQYQEIF	ISSVMMFCI	GIALIFGWEL	198
RepA	IPAEADTLIG	WVELEISHNG	TLRGYRSLE	ASLLLIIFTGL	AFTAILAVRM	180
ApdA	IPDESDDLIG	WVELDLSHSG	MLRGYRNLF	ASLLLIIPAGL	AVTALLAVRM	190
Phen	IPDEADRLIG	WVEVELSHNG	MLRGYRSLE	ASLLLIATIGL	ICTAALAVRI	190
LemA	IPAEADTLIG	WVELEISHNG	TLRGYRSLE	ASLLLIIFTGL	AFTATLAVRM	180
RpfC	MLL	YT.....	C	TGAIMAIQGE	PASPLYAVCM	62
RpfA	MRDVTTPIRN	MVDTVDRIRR	GLDSRVEGY	MLGELDMLKN	GINSWAMSET	247
BarA	MRDVTGPIRN	MVNTVDRIRR	GLDSRVEGF	MLGELDMLKN	GINSWAMSLA	248
RepA	SRTINGPLSQ	IKQAVSQLKD	GNLETRLPPPL	GSRELDDELAS	GINRMAATLQ	230
ApdA	GRTINNPLTQ	IKQAVAQLKD	GNLETRLPPPL	GSQELDELAS	GINRMASTLQ	240
Phen	SRTINSPIGQ	IKQPVAQLKD	GNLETCLAPL	GSQELDQLAS	GINRMAETLQ	240
LemA	SRTINGPMSQ	IKQAVSQLKD	GNLETRLPPPL	GSRELDDELAS	GINRMAATLQ	230
RpfC	WVTIGNGLRY	GSNYL.....	.RAATAMGSL	CF..LGAILI	SPYWLANPY.	103
RpfA	AYHEEMQONT	DQATYDLRET	LEQMEIQNVE	LDLARKEATE	AARIKSEFLA	297
BarA	AYHEEMQONI	DQATSDLRET	LEQMEIQNVE	LDLARKEATE	AARIKSEFLA	298
RepA	NAQEELQMSI	DQATEDVRQN	LETTIEIQNIE	LDLARKEATE	ASRIKSEFLA	280
ApdA	NAQEELQHSI	DQATEDVRQN	LETTIEIQNIE	LDLARKEATE	ASRIKSEFLA	290
Phen	NAQEELQHSI	DQATEDVRQN	LETTIEIQNIE	LDLARKEATE	ASRIKSEFLA	290
LemA	NAQEELQLSI	DQATEDVRQN	LETTIEIQNIE	LDLARKEATE	ASRIKSEFLA	280
RpfC	LSWGL	LLGLIAVPLY	FDSLL...RA	MTRAVREARH	ANQAKSRFLA	145
● H box						
RpfA	NMSHELRTPL	NGILGFTROT	IKTPLNTTOT	DYLLTTERS	NNLLNIINDV	347
BarA	NMSHELRTPL	NGVIGFTRLT	IKTELTPTOR	DHLNTTERS	NNLLNIINDV	348
RepA	NMSHEIRTP	NGILGFTHLL	OKSELTPROY	DYLGTEKSA	DNLLSIINEI	330
ApdA	NMSHEIRTP	NGILGFTHLL	OKSELTPROL	DYLGTEKSA	DSLLGIINEI	340
Phen	NMSHEIRTP	NGILGFTHLL	OKSELSPRHV	DYLGTEKSA	DNLLGIINEI	340
LemA	NMSHEIRTP	NGILGFTHLL	OKSELTPROF	DYLGTEKSA	DNLLSIINEI	330
RpfC	NMSHEFRTPL	NGLSGMTEVL	ATTRLDAEOK	EKLNTIQASA	RSLLSLVEEV	195

(continued on next page)

Fig. 3. Amino acid sequence alignment of RpfA with other prokaryotic two-component sensor-regulator proteins. Residues that are identical with RpfA are shaded. A darkened circle at position 301 in RpfA indicates the conserved autophosphorylated histidine residue in the sensor domain. The darkened circle at position 725 in RpfA indicates the conserved aspartic acid residue that is phosphorylated in the response regulator domain. Open circles depict other key residues conserved in two-component regulatory proteins. Conserved domains named according to their signature residues (Stock et al. 1995) are shown with boxes: H box, amino acids 295 to 312 in RpfA; N box, amino acids 401 to 422 in RpfA; D/F box, amino acids 444 to 471 in RpfA; and G box, amino acids 480 to 499 in RpfA.

Fig. 3. (continued from preceding page)

RpfA	LDFSKEAGK	IVLEDSIPFSL	HNTDDVVML	LHTAHKGL	ELTSLIQNDV	397
BarA	LDFSKEAGK	IVLESIPFPL	RSTIDEVVTI	LHSSHDKGL	ELTLNKSIV	398
RepA	LDFSKEAGK	IVLDNIPFNL	RDLIQDTLTI	LAPAAHAKOL	ELVSLVYRDT	380
ApdA	LDFSKEAGK	IVLDSIPFNL	RDLIQDTLTI	LAPAAHAKOL	ELVSLVYRDT	390
Phen	LDFSKEAGK	IVLDSVPFNL	RDLIQDTLTI	LAPAAHAKOL	ELVSLVYRDT	390
LemA	LDFSKEAGK	IVLDNIPFNL	RDLIQDTLTI	LAPAAHAKOL	ELVSLVYRDT	380
RpfC	LDISATEAGK	IRIDRRDFSL	REMIGSVNLI	LQFQARGRRL	EYGTQVADDV	245
N box						
RpfA	FEQFVGDLPLR	IQQIITNLIG	NAIKFTEQCN	IDIRV.EKRR	QELHQVLLDEV	446
BarA	PDNVIGDLPLR	LQQIITNLVG	NAIKFTENG	IDILV.EKRA	LSNTKVQIEV	447
RepA	FLALSGDLPLR	LKQIITNLVS	NAIKFTREGT	IVARA.MLED	ETEEHAQLRI	429
ApdA	PLSLVGDLPLR	LKQIITNLVS	NAIKFTREGT	IVARA.MLEE	EHEDSVQLRI	439
Phen	FLAVVGDLPLR	LKQIITNLIS	NAIKFTREGT	IVARA.MVED	EEEDSVQLRI	439
LemA	FLALSGDLPLR	LKQIITNLVS	NAIKFTREGT	IVARA.MLED	ETEEHAQLRI	429
RpfC	FDLLKGDTHA	LKQVLLNLVG	NAIKFTEEGH	VLLRVTRVSG	SAEDAVRLRF	295
D/F box						
RpfA	QIRDTGIGIA	ELQQSQLFQA	FRQADTSISR	REGGTGLGLV	ITQRFVKEMG	496
BarA	QIRDTGIGIP	ERDQSRLLFQA	FRQADASHSR	REGGTGLGLV	ITQRFVNMKG	497
RepA	SVQDTGIGLS	SQDVRLFQA	FSQADNSISR	QFGGTGLGLV	ISKRLIEQMG	479
ApdA	SIQDTGIGLS	NQDVRLFQA	FSQADNSISR	QFGGTGLGLV	ISKRLIEQMG	489
Phen	SVQDTGIGLS	NQDVRLFQA	FTEADNSISR	QFGGTGLGLV	ISKRLIEQMG	489
LemA	SVQDTGIGLS	SQDVRLFQA	FSQADNSISR	QFGGTGLGLV	ISKRLIEQMG	479
RpfC	DVEDTGIGVP	MDMRRLFQA	FEQADVGLSR	RYEGTGLGTT	IAKGLVEAMG	345
G box						
RpfA	GDISFQSELS	KGSTFWFHIT	LPLNPHAIPT	EPAYTMLQCK	HLAYVEYHPI	546
BarA	GDISFHSQPN	RGSTFWFHIN	LDLNPNIIE	GPSTQCLAGK	RLAYVEPNISA	547
RepA	GEIGVLDSTPG	EGSEFWISLN	LP.KAREDR	ETANQALEGL	RAAVLEHHDL	528
ApdA	GEIGVLDSTPG	EGSEFWISLN	LP.KTRDDAE	DLPGPPLLGR	RVAVLENHEL	538
Phen	GEIGVLDSTPG	EGSEFWISLN	LP.KTRDDVD	DLPSAPLLGR	RVAVLENHNDV	538
LemA	GEIGVLDSTPG	EGSEFWISLK	LP.KAREDK	ESLNIPLGGL	RAAVLEHHDL	528
RpfC	GSIGFKENQP	SGSVFWFELP	M.....			366
RpfA	AAQATLDILS	QTPFIVSYSP	TFEQL.....	PEGEFD	ILLGLGIPVQY	587
BarA	AAQATLDILS	ETPFEVVYSP	TFSAL.....	PPAHYD	MMLGLGIAVTF	588
RepA	ARQALEHQLE	DCGQTVVFT	NLENLLNGVT	AAHETPQAID	LVLGVT...	575
ApdA	ARQALQHOLE	DCGQVTPFN	TLEALTNGIT	GVHQSEQAID	LAVLGIT...	585
Phen	ARQALQHOLE	DCGQVTPFN	TLESALTNGIT	STHQTEQAID	LAVLGVT...	585
LemA	ARQALEHQLE	DCGQTVTFN	NLENLLNGVT	AAHETPAAID	LAVLGVT...	575
RpfC	...AIGEPLK	SSTVRVP...				380
RpfA	RNTLLDYTPR	LRDICRRSPC	VILALPSLAQ	MDAEQLKTFG	VHACLS....	633
BarA	REPLTMQHER	LAKAVSMTDF	LMLALPCHAQ	VNAEKLKQDG	IGACLL....	634
RepA	..ALEISPER	LRQHIWDLEN	LNCKVMVLCP	TTEHALFQMS	VHDVYTQLQA	623
ApdA	..TNDMSPER	LSQHIWDLEH	LGCKVLVLCP	TTEQTLFHL	VPNPNSQLQA	633
Phen	..ANDIPPER	LNQHLWDLEH	LGCKVLVLCP	TTEQMLFNQS	VPNPNSQLQA	633
LemA	..ALEISPER	LRQHIWDLEN	LNCKVMVLCP	TTEHALFQLA	VHDVYTQLQA	623
RpfC			..TGALVDAP	EELESSNIIA	FSNPFLRHRA	408
O						
RpfA	KPLAASRLLP	LLQDSTLFQF	SFLPDDTASH	QSAVRHLARL	PLRVMAVDDN	683
BarA	KPLTPTRLPP	ALTE.....	..FCHHKQNT	LLPVTDESKL	AMRVMAVDDN	676
RepA	KPACNRKLQK	ALSE.....	..LVAPRAVR	TDVALPLSSR	APRVLCVDDN	665
ApdA	KPACTRKLRR	ALSD.....	..LVTPRRAR	SEPETLSSR	APRVLCVDDN	675
Phen	KTPCTRKLRR	ALAD.....	..LISPPVAQ	RTR.EPLSSR	APRVLCVDDN	674
LemA	KPACTRKLQK	ALSE.....	..LIAPRAVR	ADIGPPLSSR	APRVLCVDDN	665
RpfC	RV.....			R	SMRMLVADDE	421

(continued on next page)

Fig. 3. (continued from preceding page)

Rp fA	PANLKLIGTL	LEEQVETIIL	CESGQD	IAQ	AELNQCDIIL	MDIQMPGMDG	733
BarA	PANLKLIGAL	LEDVMQHVEL	CDSGHQ	VER	AKQMPFDLIL	MDIQMPDMDG	726
RepA	PANLLIVQTL	LEDMGAEVVA	VDG GYA	VSA	VQQEAFDLVL	MDVQMPGMDG	715
Ap dA	PANLLLIQTL	LEDMGAKVLA	VDN GYA	LNA	IQTEPFDLVM	MDVQMPGMDG	725
PheN	PANLLIVQTL	LEDMGAKVLA	VES GYA	IDA	VKQETFDLVL	MDVQMPGMDR	724
LemA	PANLLIVQTL	LEDMGAEVVA	VEG GYA	VNA	VQQEAFDLVL	MDVQMPGMDG	715
Rp fC	EANRMVLQRI	LEKAGHKVLC	VNGAEQVLDA	MAEEDYDAVI	VDLEMPGMNG		471
Rp fA	ICASELIRQI	...PHHATIP	IIAVTAQTMT	GEREHLIRSG	MDDYIAKPID		780
BarA	IRACELIHQL	...PHQQQTF	VIATTAHAMA	GQKEKLLGAG	MSDYIAKPIE		773
RepA	CQATEAIRAW	ETERNQTSLE	IVALTAHAMA	NEKRSLLQSG	MDDYITKPIE		765
Ap dA	RKSTEAIRQW	ESERHGTPLP	IVALTAHAMA	NEKRALLQSG	MDDYITKPIE		775
PheN	RQSTEAIRQW	ESERHGTPLP	VVALTAHAMA	NEKRALLQSG	MDDYITKPIE		774
LemA	RQATEAIRAW	EAERNQSSLE	IVALTAHAMA	NEKRSLLQSG	MDDYITKPIE		765
Rp fC	LDMLKQLRVM	QASGMRY.TP	VVVLSADVTP	EAIRACEQAG	ARAFIAKPIV		520
Rp fA	EQMKSVLTR	HARQDPLKRN	RGE..MTGLL	NEHDDSQLSL	DWALAQQQAA		828
BarA	BERLHNLLLR	YKPGSGIS.S	RVV..TPEVN	EIVVNFNATL	DWQLALRQAA		820
RepA	ERQLAQVVLK	WT.GLALRNP	IPE..RQTES	SNAHVGPLVL	DHEEGLRIAA		812
Ap dA	ERQLAQVVLK	WT.GLALRNQ	GPE..RASER	FELGLELQVL	DQDEGLRIAA		822
PheN	ERQLAQVVLK	WT.GLALRNP	GPE..RGNDN	LGHGVQLLVL	DHEEGL.AAA		820
LemA	ERQLAQVVLK	WT.GLALRNP	APE..RQNEA	LEVHVGPLVL	DHEEGLRIAA		812
Rp fC	AAKLLDNFGR	SGSEHPAVGD	AATTVQVATS	FEGVLDSSVL	DELAALGMGE		570
Rp fA	NKPELGRDLI	QMLDFLPEV	QRKIENVLKG	QTDDDIIELV	HKLHGSCSYS		878
BarA	GKTDLARDML	QMLDFLPEV	RNKVEEQLVG	ENPEGLVDLI	HKLHGSCGYS		870
RepA	GKPDLAADML	AMLPAASLDAD	REAIRAARAN	DDIQGLIERV	HRLHGATRYC		862
Ap dA	GKADLAADML	AMLPAASLDAD	REAIKAARAA	NDQNALIERV	HRLHGATRYC		872
PheN	NKADLAADML	AMLPAASLEAD	RLAITVAREA	KDNNALIERI	HRLHGATRYC		870
LemA	GKADLAADML	AMLPAASLDAD	REAIRVARAN	QDVHALIERI	HRLHGATRYC		862
Rp fC	...EFERQFV	RQCTDDAQNC	VGDIERDGTG	SDWEQLRESA	HALRGVASNL		617
Rp fA	GVP.LQPICR		YLBQQLRKGV	HASDLEPEWL	ELLDEIDNVN		917
BarA	GVPRMKNLCQ		LLEQQLRSGT	KEEDLEPELL	ELLEDMDNVA		910
RepA	GVPQLRTACQ		RAETTLKQN.	.APHTEDALD	DLDKAITRLE		900
Ap dA	GVPQLRAACQ		RSETTLKQE.	.DAKAFALD	ELDHAIGRLA		910
PheN	GVAELRAACQ		RAETTLKQD.	.DAQGHGALD	ELDMAIG..A		906
LemA	GVPQLRSACQ		RAETTLKQN.	.APHTEEALN	DLDKAITRLE		900
Rp fC	GLAQVASSGG	ELMRMADWQL	QAEWRRLST	LREQLKAGKD	ALDARVQGVK		667
Rp fA	RAAQPHINPR	LP					929
BarA	REASKILG						918
RepA	AEARVTA						907
Ap dA	AEARTNA						917
PheN	AGQ						909
LemA	AEARVMA						907
Rp fC	DGECSPRSNE						677

tional pathogenicity genes. The use of *cel-* and *prt-*reporter gene fusions is one way to genetically dissect this complex system for pathogenicity gene activation in *E. carotovora* subsp. *carotovora*. The environmental signal or signals that trigger the activation of RpfA are undefined at present, and their elucidation could prove valuable for devising novel disease-control strategies that would block the activation of

E. carotovora subsp. *carotovora* pathogenicity factors such as cellulase and protease enzymes.

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LITERATURE CITED

- Andro, T., Chambost, J.-P., Kotoujansky, A., Cattaneo, J., Bertheau, Y., Barras, F., Van Gijsegem, F., and Coleno, A. 1984. Mutants of *Erwinia chrysanthemi* defective in secretion of pectinase and cellulase. *J. Bacteriol.* 160:1199-1203.
- Boccardo, M., Dirole, A., Rouve, M., and Kotoujansky, A. 1988. The role of individual pectate lyases of *Erwinia chrysanthemi* strain 3987 in pathogenicity on Saintpaulia plants. *Physiol. Mol. Plant Pathol.* 33:95-104.
- Boyer, H. W., and Roulland-Dussoix, D. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* 41:459-472.
- Brennan, R. G., and Matthews, B. W. 1989. The helix-turn-helix DNA binding motif. *J. Biol. Chem.* 264:1903-1906.
- Castillo, B. A., Olfson, P., and Casadaban, M. J. 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. *J. Bacteriol.* 158:448-495.
- Chatterjee, A. K., and Vidaver, A. K. 1986. Advances in Plant Pathology. Vol. 4. Genetics of Pathogenicity Factors: Application to Phytopathogenic Bacteria. P. S. Ingram and P. H. Williams, eds. Academic Press, London.
- Chatterjee, A., Cui, Y., Liu, Y., Dumenyo, C. K., and Chatterjee, A. K. 1995. Inactivation of *rsmA* leads to overproduction of extracellular pectinases, cellulases, and proteases in *Erwinia carotovora* subsp. *carotovora* in the absence of the starvation/cell density-sensing signal, *N*-(3-oxohexanoyl)-L-homoserine lactone. *Appl. Environ. Microbiol.* 61:1959-1967.
- Collmer, A., and Keen, N. T. 1986. The role of pectic enzymes in plant pathogenesis. *Annu. Rev. Phytopathol.* 24:383-409.
- Cooper, J. C., and Salmond, G. P. C. 1993. Molecular analysis of the major cellulase (*celV*) of *Erwinia carotovora*: Evidence for an evolutionary "mix-and-match" of enzyme domains. *Mol. Gen. Genet.* 241:341-350.
- Corbell, N., and Loper, J. E. 1995. A global regulator of secondary metabolite production in *Pseudomonas fluorescens* Pf-5. *J. Bacteriol.* 177:6230-6236.
- Cui, Y., Chatterjee, A., Liu, Y., Dumenyo, C. K., and Chatterjee, A. K. 1995. Identification of a global repressor gene, *rsmA*, of *Erwinia carotovora* subsp. *carotovora* that controls extracellular enzymes, *N*-(3-oxohexanoyl)-L-homoserine lactone, and pathogenicity in soft-rotting *Erwinia* spp. *J. Bacteriol.* 177:5108-5115.
- Ditta, G. W., Stanfield, S., Corbin, D., and Helinski, D. R. 1980. Broad host range DNA cloning system for gram-negative bacteria: Construction of a gene bank of *Rhizobium meliloti*. *Proc. Natl. Acad. Sci. U.S.A.* 77:7347-7351.
- Frischauf, A.-M., Lehrach, H., Poustka, A., and Murray, N. 1983. Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.* 170:827-842.
- Grewal, S. I. S., Han, B., and Johnstone, K. 1994. Regulation of toxin synthesis and phenotypic variation in *Pseudomonas tolaasii*, cause of Brown Blotch Disease of mushrooms. Pages 25-32 in: Advances in Molecular Genetics of Plant-Microbe Interactions. M. J. Daniels, J. A. Downie, and A. E. Osbourn, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
- Grewal, S. I. S., Han, B., and Johnstone, K. 1995. Identification and characterization of a locus which regulates multiple functions in *Pseudomonas tolaasii*, the cause of brown blotch disease of *Agaricus bisporus*. *J. Bacteriol.* 177:4658-4668.
- Handa, A. K., Bressan, R. A., Korty, A. G., Jayaswal, R. K., and Charles, D. J. 1987a. Isolation and characterization of pectinolytic nonpathogenic mutants of *Erwinia carotovora* subsp. *carotovora*. Pages 212-217 in: Plant Pathogenic Bacteria. E. L. Civerolo, A. Collmer, R. E. Davis, and A. G. Gillaspie, eds. Martinus Nijhoff, Dordrecht, Netherlands.
- Handa, A. K., Bressan, R. A., Lee, L., Charles, D. J., Jayaswal, R. K., Chiu, J., and Bennetzen, J. L. 1987b. Characterization of pathogenicity genes of *Erwinia carotovora*. Pages 67-72 in: Molecular Genetics of Plant-Microbe Interactions. D. P. S. Verma and N. Birsson, eds. Martinus Nijhoff, Dordrecht, Netherlands.
- Hinton, J. C. D., Sidebotham, J. M., Hyman, L. J., Perombelon, M. C. M., and Salmond, G. P. C. 1989. Isolation and characterization of transposon induced mutants of *Erwinia carotovora* subsp. *atroseptica* exhibiting reduced virulence. *Mol. Gen. Genet.* 217:141-148.
- Hrabak, E. M., and Willis, D. K. 1992. The *lemA* gene required for pathogenicity of *Pseudomonas syringae* pv. *syringae* on bean is a member of a family of two-component regulators. *J. Bacteriol.* 174:3011-3020.
- Jayaswal, R. K., Bressan, R. A., and Handa, A. K. 1984. Mutagenesis of *Erwinia carotovora* subsp. *carotovora* with bacteriophage MuD1(Ap^llac cts62): Construction of *his-lac* gene fusions. *J. Bacteriol.* 158:764-766.
- Jayaswal, R. K., Bressan, R. A., and Handa, A. K. 1985. Effects of a mutation that eliminates UDP glucose-pyrophosphorylase on the pathogenicity of *Erwinia carotovora* subsp. *carotovora*. *J. Bacteriol.* 164:473-476.
- Jones, S., Yu, B., Bainton, N. J., Birdsall, M., Bycroft, B. W., Chhabra, S. R., Cox, A. J. R., Golby, P., Reeves, P. J., Stephens, S., Winson, M. K., Salmond, G. P. C., Stewart, G. S. A. B., and Williams, P. 1993. The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. *EMBO J.* 12:2477-2482.
- Keen, N. T., Dahlbeck, D., Staskawicz, B., and Belser, W. 1984. Molecular cloning of pectate lyase genes from *Erwinia chrysanthemi* and their expression in *Escherichia coli*. *J. Bacteriol.* 159:825-831.
- Kotoujansky, A. 1987. Molecular genetics of pathogenesis by soft-rot *erwinias*. *Annu. Rev. Phytopathol.* 25:405-430.
- Kyostio, S. R. M., Cramer, C. L., and Lacy, G. H. 1991. *Erwinia carotovora* subsp. *carotovora* extracellular protease: Characterization and nucleotide sequence of the gene. *J. Bacteriol.* 173:6537-6546.
- Kyte, J., and Doolittle, R. F. 1982. A simple method for displaying hydropathic character of a protein. *J. Mol. Biol.* 157:105-132.
- Laville, J., Voisard, C., Keel, C., Maurhofer, M., Deafo, G., and Haas, D. 1992. Global regulation in *Pseudomonas fluorescens* mediating antibiotic synthesis and suppression of black root rot of tobacco. *Proc. Natl. Acad. Sci. U.S.A.* 89:1972-1977.
- Liao, C.-H., McCallus, D. E., and Fett, W. F. 1994. Molecular characterization of two gene loci required for production of the key pathogenicity factor pectate lyase in *Pseudomonas viridiflava*. *Mol. Plant-Microbe Interact.* 7:391-400.
- Liu, Y., Murata, H., Chatterjee, A., and Chatterjee, A. K. 1993. Characterization of a novel regulatory gene *aepA* that controls extracellular enzyme production in the phytopathogenic bacterium *Erwinia carotovora* subsp. *carotovora*. *Mol. Plant-Microbe Interact.* 6:299-308.
- Murata, H., McEvoy, J. L., Chatterjee, A., Collmer, A., and Chatterjee, A. K. 1991. Molecular cloning of an *aepA* gene that activates production of extracellular pectolytic, cellulolytic, and proteolytic enzymes in *Erwinia carotovora* subsp. *carotovora*. *Mol. Plant-Microbe Interact.* 4:239-246.
- Murata, H., Chatterjee, A., Liu, Y., and Chatterjee, A. K. 1994. Regulation of the production of extracellular pectinase, cellulase, and protease in the soft rot bacterium *Erwinia carotovora* subsp. *carotovora*: Evidence that *aepH* of *E. carotovora* subsp. *carotovora* 71 activates gene expression in *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *Escherichia coli*. *Appl. Environ. Microbiol.* 60:3150-3159.
- Nagasawa, S., Tokishita, S., Aiba, H., and Mizuno, T. 1992. A novel sensor-regulator protein that belongs to the homologous family of signal-transduction proteins involved in adaptive responses in *Escherichia coli*. *Mol. Microbiol.* 6:799-807.
- Parkinson, J. S., and Kofoid, E. C. 1992. Communication modules in bacterial signaling proteins. *Annu. Rev. Genet.* 26:71-112.
- Perombelon, M. C. M. 1987. Pathogenesis by pectolytic *erwinias*. Pages 109-120 in: Plant Pathogenic Bacteria. E. L. Civerolo, A. Collmer, R. E. Davis, and A. G. Gillaspie, eds. Martinus Nijhoff, Dordrecht, Netherlands.
- Perombelon, M. C. M., and Kelman, A. 1980. Ecology of the soft rot *erwinias*. *Annu. Rev. Phytopathol.* 18:361-387.
- Pirhonen, M., Saari-Lahti, H., Karlsson, M.-B., and Palva, E. T. 1991. Identification of pathogenicity determinants of *Erwinia carotovora* subsp. *carotovora* by transposon mutagenesis. *Mol. Plant-Microbe Interact.* 4:276-283.
- Pirhonen, M., Flego, D., Heikinheimo, R., and Palva, E. T. 1993. A small diffusible signal molecule is responsible for the global control

- of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. EMBO J. 12:2467-2476.
- Rich, J. J., Kinscherf, T. G., Kitten, T., and Willis, D. K. 1994. Genetic evidence that the *gacA* gene encodes the cognate response regulator for the *lemA* sensor in *Pseudomonas syringae*. J. Bacteriol. 176:7468-7475.
- Saari-Lahti, H. T., Henrissat, B., and Palva, E. T. 1990. *celS*: A novel endoglucanase identified from *Erwinia carotovora* subsp. *carotovora*. Gene 90:9-14.
- Salmond, G. P. C., Bycroft, B. W., Stewart, G. S. A. B., and Williams, P. 1995. The bacterial "enigma": Cracking the code of cell-cell communication. Mol. Microbiol. 16:615-624.
- Staskawicz, B. J., Dahlbeck, D., Keen, N., and Napoli, C. 1987. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 169:5789-5794.
- Stock, J. B., Surette, M. G., Levit, M., and Park, P. 1995. Two-component signal transduction systems: Structure-function relationships and mechanisms of catalysis. Pages 25-51 in: Two-Component Signal Transduction. J. A. Hoch and T. J. Silhavy, eds. American Society for Microbiology, Washington, DC.
- Tang, J. L., Gough, C. L., Barber, C. E., Dow, J. M., and Daniels, M. J. 1987. Molecular cloning of protease gene(s) from *Xanthomonas campestris* pv. *campestris*: Expression in *Escherichia coli* and role in pathogenicity. Mol. Gen. Genet. 210:443-448.
- Tang, J. L., Liu, Y.-N., Barber, C. E., Dow, J. M., Wootton, J. C., and Daniels, M. J. 1991. Genetic and molecular analysis of a cluster of *rpf* genes involved in positive regulation of synthesis of extracellular enzymes and polysaccharide in *Xanthomonas campestris* pathovar *campestris*. Mol. Gen. Genet. 226:409-417.