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MINI-REVIEW

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Thermoregulated expression of virulence factors in plant-associated bacteria

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Abstract Pathogenic bacteria with habitats inside and outside a given host react to changes in environmental parameters by synthesizing gene products specifically needed during pathogenic or saprophytic growth. Temperature effects have been investigated in detail for pathogens of warm-blooded hosts, and major principles governing the temperature-sensing mechanism have been uncovered. Generally, transcription of virulence genes in these pathogens is induced at higher temperatures (37–41 °C), which are typical for body cavities and host tissues. However, effects of temperature on virulence determinants in plant pathogenic bacteria have not been focused on in detail. Interestingly, almost all virulence genes of plant pathogenic bacteria studied with respect to temperature exhibit increased transcription at temperatures well below the respective growth optima. This includes virulence determinants such as those directing bacteria-to-plant gene transfer, plant cell-wall-degrading enzymes, phytotoxins, ice nucleation activity, exopolysaccharide production, and the type III protein secretion machinery. Although many of the studied phytopathogens cause "cold-weather" diseases, the ecological rationale for this phenomenon remains to be studied in detail. This mini-review summarizes our current knowledge on thermoregulation of cellular processes taking place in bacterial phytopathogens in response to temperature changes. Since the temperature range of interest is different from that relevant to pathogens of mammals, one envisions novel principles of thermo-sensing in bacteria interacting with plants.

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Introduction

As a major environmental factor, temperature has profound effects on cellular processes in all organisms. Due to their high surface-to-volume ratio, prokaryotes have to stringently cope with temperature changes and adapt to a given temperature regime. These processes are carried out by modulation of their primary and secondary metabolism using a wide range of molecular mechanisms. An increase in temperature generally gives rise to accelerated enzymatic activities.

Bacteria and archaea have occupied all life-supporting ecological niches and have adapted to the respective temperatures, allowing them to be classified as psychrophilic, mesophilic, or thermophilic microorganisms. Dynamic temperature gradients govern most microbial habitats in terms of space and time. Adaptations of prokaryotes to extreme temperatures will not be discussed in this review. Instead, adaptive mechanisms by which mesophilic bacteria respond to minor temperature changes will be analyzed in detail.

While some of the molecular mechanisms controlling the differential expression of virulence determinants in bacteria pathogenic to humans and animals have been studied in detail, little is known about the signaling pathways and mode of thermoregulation in bacteria that are not associated with mammals. This review specifically focuses on temperature effects in plant-associated bacteria and highlights some of the best-studied systems (Table 1). The majority of these systems show increased expression of genes coding for virulence determinants and/or an increased secretion of proteins at reduced temperatures such as 16–24 °C. Although the ecological rationale for this is far from being understood, the phenomenon could be explained by the fact that most plant pathogenic bacteria re-

Table 1 Thermoresponsive virulence determinants in plant pathogenic bacteria

Pathogen	Disease	Virulence factor	Temperature Induced:	Temperature Repressed:	Induced at level of	Reference
Agrobacterium tumefaciens	Crown gall	T-DNA transfer	20 °C	>32 °C	Two-component system	Jin et al. 1993
			19 °C	28 °C	Assembly of T-pilus	Banta et al. 1998; Lai and Kado 1998
Erwinia chrysanthemi	Soft rot	Pectinase(s)	25 °C	37 °C	Transcription	Hugovieux-Cotte-Pattat et al. 1992
Erwinia carotovora	Soft rot	Cellulase(s)	27 °C	30.5 °C	Unknown	Lanham et al. 1991
Erwinia amylovora	Fire blight	Type III *hrp* system	18 °C	28 °C	Transcription	Wei et al. 1992
		Levan formation	18 °C	28 °C	Transcription	Bereswill et al. 1997
		Amylovoran synthesis	28 °C	37 °C	Transcription	Kelm et al. 1997
Pseudomonas syringae	Leaf spot diseases	Coronatine	18 °C	28 °C	Two-component system protein stability	Ullrich et al. 1995; Budde et al. 1998; Rohde et al. 1999
		Type III *hrp* system	18 °C	28 °C	Assembly of Hrp pilus	Van Dijk et al. 1999
		Phaseolotoxin	18 °C	28 °C	Repression of transcription	Rowley et al. 1993; Rowley et al. 2000
		Ice nucleation activity	16 °C	24 °C	Transcription	Nemececk-Marshall et al. 1993
		Alginate synthesis	28 °C	18 °C	Transcription heat shock response	Penaloza-Vazquez et al. 1997; Keith and Bender 1999
		Levan formation	18 °C	28 °C	Protein secretion	Li and Ullrich 2001

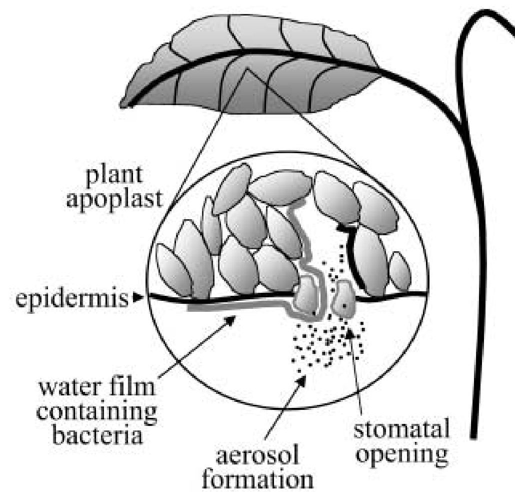

Fig. 1 Possible ecological rationale for the effects of temperature on the virulence of pathogenic bacteria that infect plant leaves. Due to the high humidity and lower temperature of the plant's surface and interior as compared to the ambient air temperature, water films and aerosols form which may foster the penetration of phytopathogens into the plant's apoplast where infection can proceed

quire water films or aerosols for the efficient infection of their host plants. Such water films and high humidity predominantly occur when the air temperature or the temperature of the plant surface is low (Fig. 1). It is interesting to note that many plant pathogenic bacteria exhibit a stronger virulence at lower temperatures although their optimal growth temperatures usually range from 25 to 30 °C. Only a few reported examples provide evidence for the opposite effect, such as the tropical pathogen *Ralstonia solanacearum*. This pathogen causes the so-called blood disease on bananas and its virulence is more dominant at higher temperatures such as 35 °C (Hayward 1991).

For the sake of comprehensiveness, thermoregulation in pathogens of mammals and global aspects of heat- and cold-shock responses in prokaryotes will only briefly be introduced. For more detailed information on these fascinating topics, please refer to excellent reviews published recently (Hurme and Rhen 1998; Konkel and Tilly 2000; Phadtare et al. 1999; Yura and Nakahigashi 1999).

Thermoregulated gene expression in pathogens of mammals

Pathogens of human and animals such as representatives of the genera *Escherichia*, *Yersinia*, *Shigella*, *Salmonella*, *Bordetella*, *Borrelia*, and *Vibrio* sense the interior of the warm-blooded host by various environmental parameters including body temperature (37–41 °C), osmolarity, pH, iron levels, and host-borne signal molecules (Konkel and Tilly 2000; Mekalanos 1992). The temperature inside mammals remains relatively constant, making it a reliable signal for the onset of virulence determinant synthesis. By a stringent temperature signal induction, the energy-con-

suming virulence gene expression is economized and proceeds only at the time and location of infections. In this respect numerous pathogen systems have been studied in detail and five major regulatory principles that determine thermoregulation in pathogens of mammals have been recognized: DNA supercoiling, changes of the mRNA secondary structure, modulation of enzymatic activity of proteins with coiled-coil structures, interference of the global heat shock response with virulence gene expression, and signal transduction by two-component regulatory systems (Hurme and Rhen 1998).

The super-helical tension of circular DNA and the stability of stem-loop structures within mRNA molecules strongly depend on temperature, thus allowing or preventing either the binding of transcriptional regulator proteins to their target DNA or the translation of the mRNA. Likewise, the coiled-coil structures in some proteins termed molecular "thermometers" refold differently at different temperatures thus modulating the enzymes' activities in DNA binding or protein-protein interactions. Heat shock proteins such as the DNA-binding protein H-NS or chaperones that are synthesized at elevated temperatures modulate the level of gene expression or foster the proper folding of proteins involved in pathogenicity, respectively (Hurme and Rhen 1998). Finally, in *Bordetella pertussis* the two-component regulatory system BvgS/BvgA coordinates the thermoresponsive expression of virulence genes. The histidine protein kinase BvgS might function as a molecular thermometer but its mode of action remains to be elucidated (Konkel and Tilly 2000).

Heat and cold shock response in prokaryotes

Microorganisms respond to abrupt temperature shifts by a rapid accumulation of so-called heat or cold shock proteins, most of which have functions as chaperones, proteases, and DNA- or ribosome-binding proteins (Yura and Nakahigashi 1999; Phadtare et al. 1999). While a few heat shock proteins have been implicated in thermoregulation of virulence genes in human and animal pathogens (see above), most of the temperature shock proteins have a more general function in maintaining correct protein folding, membrane fluidity, and protein synthesis. Disruption of genes encoding for heat or cold shock proteins leads to a dramatic decrease of bacterial survival at marginal and life-threatening temperatures. These proteins are transiently synthesized following a sudden shift in temperature of ≥15 °C. In part, this feature might explain why temperature shock proteins are of minor importance during the long-term adaptation processes bacteria undergo when their habitat temperature changes slightly or when bacteria are translocated to new habitats.

DNA transfer by *Agrobacterium tumefaciens*

Knowledge of the transfer of Ti-plasmid-borne DNA from *Agrobacterium tumefaciens*, the causal agent of crown gall disease, to plant cells has tremendously accelerated plant genetics, and the molecular mechanisms for this DNA transfer have been studied in detail (Kado 1998). Besides plant-borne signals such as phenolic compounds and acetosyringone, low temperature significantly induces the expression of virulence (*vir*) genes (Jin et al. 1993) and the assembly of the T-DNA transfer machinery, a type IV pilus-like multi-component apparatus (Banta et al. 1998; Lai and Kado 1998). This explains why symptoms of crown gall disease predominantly occur at low temperatures. Transcription of the *vir* operon, required for the assembly of the T-DNA transfer machinery, is regulated by the two-component regulatory system VirA/VirG (Jin et al. 1993). The response regulator VirG binds to its target DNA when phosphorylated by the histidine kinase VirA, which prior to this step undergoes autophosphorylation upon reception of the external signals. At temperatures above 32 °C, VirA is inactive, and point mutations in the N-terminal receiver region of VirA led to its constitutive (temperature-independent) activation (Jin et al. 1993). Therefore, VirA may constitute a thermo-sensor but its mode of action remains to be determined. Moreover, Banta et al. (1998) and Lai and Kado (1998) showed that the assembly and stability of the T-DNA transfer complex composed of 11 VirB proteins is optimal at temperatures around 20 °C and is significantly reduced at elevated temperatures.

Secretion of virulence determinants

Pectinases of *Erwinia* species causing soft rot

The plant pathogens *Erwinia chrysanthemi* and *Erwinia carotovora* cause soft rot on numerous plants and depolymerize pectin from the plant cell wall using several extracellular pectinases (Pel) whose expression is substrate-inducible and subject to complex regulation (Hugovieux-Cotte-Pattat et al. 1996). Temperature is a primary environmental factor affecting the virulence of pectinolytic *Erwiniae*. Transcriptional activation of the *pel* genes depends on growth temperature, with maximal levels of expression at 25 °C and up to 15-fold lower transcription at 37 °C (Hugovieux-Cotte-Pattat et al. 1992). Out of seven genes encoding pectinases in *E. chrysanthemi*, the genes for two basic isoenzymes, *pelD* and *pelE*, show the most pronounced thermoregulation. The products of these two genes are secreted at high rates, efficiently macerate plant tissue, and may play a major role during the infection process (Hugovieux-Cotte-Pattet et al. 1996). Similar observations have been made for extracellular cellulases in *E. carotovora* (Lanham et al. 1991), demonstrating that common regulatory principles may govern the secretion of virulence determinants in different soft-rot-causing plant pathogens.

Type III protein secretion in *Erwinia amylovora* and *Pseudomonas syringae*

Phytopathogenic bacteria use a widely conserved type III protein secretion apparatus termed the Hrp (hypersensitive response and pathogenicity) system to translocate so-called avirulence (*avr*) gene products into the plant cell thereby causing either a hypersensitive response on non-host plants or pathogenicity on susceptible host plants. Opportunistic pathogens such as the causal agent of fire blight, *E. amylovora*, and the bacterial blight pathogen, *Pseudomonas syringae*, which preferentially infect their hosts under conditions of cold and humid weather, secrete Avr proteins via the Hrp system in a temperature-dependent manner. Wei et al. (1992) showed that expression of *hrp* genes in *E. amylovora* proceeded at 18 °C and was significantly reduced at 28 °C, the optimal growth temperature of this bacterium. Likewise, secretion of *avr* gene products via the Hrp system of *P. syringae* is optimal at 18 °C and decreases at 28 °C (Van Dijk et al. 1999). In analogy to the above-mentioned T-DNA transfer machinery of *A. tumefaciens*, it is tempting to speculate that the energy-consumptive assembly of the multi-component Hrp protein complex of *P. syringae* is controlled by temperature so that it may proceed only under conditions favorable for infection.

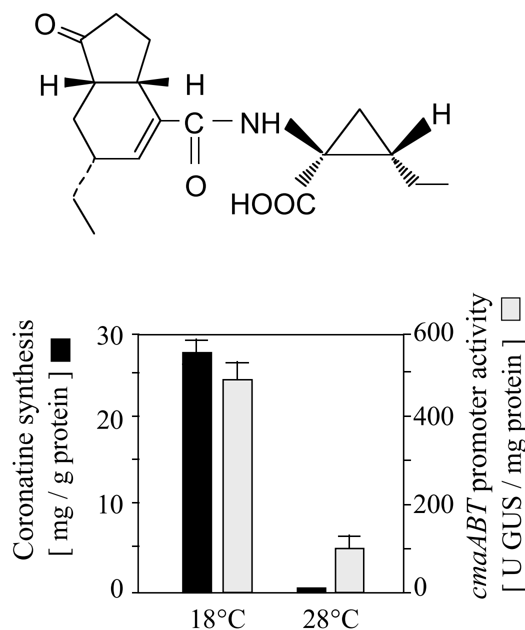

Fig. 2 Structure of the phytotoxin coronatine (COR) consisting of a polyketide moiety (*left*) and a cyclized isoleucine derivative (*right*) which are fused to each other by an amide bond (*top*). Temperature-dependent synthesis of coronatine and thermoresponsive expression of the coronatine biosynthetic *cmaABT* operon as quantified by a transcriptional fusion of the *cmaABT* promoter region to a promoterless reporter gene, *uidA*, encoding ß-glucuronidase (GUS) (*bottom*)

Phytotoxin synthesis in *Pseudomonas syringae*

Coronatine

The chlorosis-inducing and jasmonate-mimicking polyketide phytotoxin coronatine (Fig. 2A) is produced by the bacterial blight pathogen *P. syringae* in a temperature-dependent manner (Fig. 2B) (Budde et al. 1998; Palmer and Bender 1993). The gene cluster required for its biosynthesis is plasmid-borne and consists of two biosynthetic operons and three regulatory genes, *corS*, *corR*, and *corP* (Bender et al. 1996). Transcription of both biosynthetic operons and *corS* is thermoresponsive, with highest gene expression at 18 °C and low basal levels of expression at 28 °C, the optimal growth temperature for *P. syringae* (Fig. 2B). Additionally, decreased stability of coronatine biosynthetic enzymes contributes to the lack of coronatine production at 28 °C (Budde et al. 1998). A modified two-component system consisting of the histidine kinase CorS and two response regulator proteins, CorR and CorP, controls thermoresponsive coronatine gene expression (Fig. 3), and all three regulatory proteins are essential for coronatine synthesis (Ullrich et al. 1995; Wang et al. 1999). While CorR contains a classical helix-turn-helix DNA-binding domain and binds to DNA upstream of both coronatine biosynthetic operons (Peñaloza-Vázquez and Bender 1998; Wang et al. 1999), CorP lacks such a motif and is believed to have a modulatory function. DNA binding of CorR is optimal at 18 °C and requires functional CorS, which initially might be auto-phosphorylated at 18 °C and then activates CorR by phosphorylation (Rangsawamy and Bender 2000; Wang et al. 1999). CorR also positively controls expression of *corS*; therefore, the signal transduction pathway is characterized by auto-induction and signal amplification (Fig. 3). Taken together, these data allow a function of CorR as a repressor of coronatine gene expression to be ruled out. The mode of temperature sensing by CorS remains speculative, but preliminary results from our laboratory suggested that in its hydrophobic N-terminus CorS contains six trans-membrane domains from which one might „flip“ into the periplasm at 28 °C. Since the respective trans-membrane domain has a similarly low hydropathy index as the catalytic histidine (H)-box essential for CorS auto-phosphorylation, we speculate that the H-box might be inserted into the bacterial membrane at elevated temperatures thereby disabling its auto-phosphorylation activity. The latter hypothesis is in line with all phenotypic observations at 28 °C: lack of CorR activity, low transcriptional activation, and no coronatine biosynthesis. Our hypothesis could help to explain the thermo-sensing mechanism by CorS and raises the interesting question whether the degree of fatty acid saturation in the inner membrane might trigger conformational changes in CorS in dependence of temperature.

Phaseolotoxin

The causal agent of halo blight of beans, *P. syringae* pv. *phaseolicola*, synthesizes another chlorosis-inducing phy-

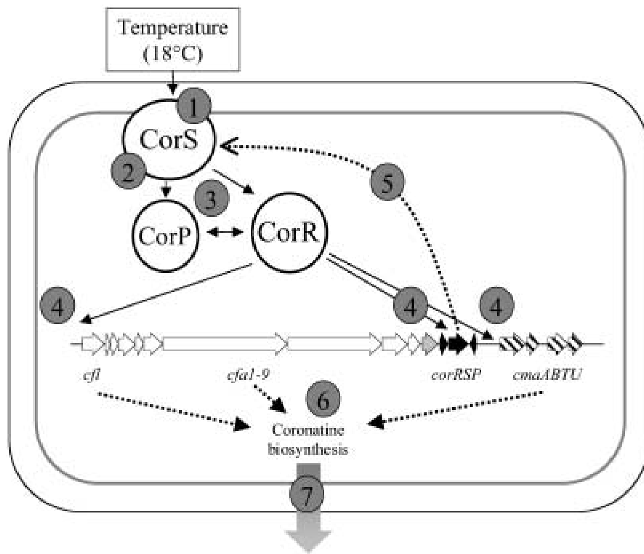

Fig. 3 Model for temperature signal reception and transduction by the modified two-component system CorSRP. Upon reception of the temperature signal by CorS (*1*), autophosphorylation of a conserved histidyl residue of CorS (*2*) may lead to a subsequent phosphorylation of aspartyl residues of the cognate response regulators CorR and CorP (*3*). In contrast to CorP, which lacks a typical helix-turn-helix motif indicative for DNA binding, phosphorylated CorR then binds to three promoter regions within the coronatine biosynthetic gene cluster (*4*). Binding to the *corS* upstream region may lead to an increased synthesis of CorS (*5*) thus amplifying the signal. Binding of CorR to the upstream regions of *cfl*/*cfa1–9* and *cmaABT* induces synthesis of biosynthetic enzymes required for coronatine biosynthesis (*6*) and subsequent secretion of coronatine from the bacterial cell (*7*)

totoxin, the modified tri-peptide phaseolotoxin, which inhibits ornithine carbamoyltransferase of the host plant. Interestingly, biosynthesis of phaseolotoxin also proceeds in a temperature-dependent manner, with maximal yields produced at 18 °C and no phytotoxin synthesized at 28 °C. Rowley et al. (1993) showed that thermoregulation of phaseolotoxin synthesis is mediated by repression of biosynthetic genes at elevated temperatures. A 24.4-kb genomic DNA fragment, which does not harbor any phaseolotoxin biosynthetic genes, abolished thermoregulation and led to constitutive phaseolotoxin synthesis. A 485-bp sub-clone from this fragment containing protein-binding motifs was used in mobility-shift assays. These revealed the presence of protein(s) in *P. syringae* pv. *phaseolicola* grown at 28 °C that specifically bound to the DNA fragment. It was proposed that the binding protein is a repressor that is "titrated" by multiple copies of the 485-bp fragment, thus allowing expression of phaseolotoxin genes (Rowley et al. 1993). Recently, Rowley et al. (2000) reported that a single site within the 485-bp fragment is protected from DNAse I cleavage by the above-mentioned repressor in the 28 °C protein extract. A toxin resistance determinant, the phaseolotoxin-resistant ornithine carbamoyltransferase encoded by the *argK* gene, is also produced by *P. syringae* pv. *phaseolicola* at 18 °C but not at 28 °C. A 492-bp upstream fragment from *argK* forms complexes with the repressor protein from 28 °C crude extracts (Rowley et al. 2000). A 260-bp fragment from the repressor-binding fragment identified by Rowley et al. (1993) cross-competed with the *argK* upstream fragment, indicating that the same protein binds to nucleotides in both fragments. The nature of this repressor remains to be determined.

Additional virulence or fitness factors

While the above-mentioned model systems have been studied in some detail, many other virulence determinants remain to be analyzed with respect to temperature effects. Frost injury on citrus plants caused by *P. syringae* is mediated by the activity of a bacterial ice nucleation protein. As expected, the respective *inaZ* gene coding for this protein is expressed at maximal rate at 16 °C and is only minimally transcribed at 24 °C (Nemecek-Marshall et al. 1993). Interestingly, further lowering the incubation temperature does not further increase *inaZ* expression. A unique situation with respect to temperature exists for exopolysaccharide (EPS) synthesis in *P. syringae*. This pathogen produces two major EPS, alginate and levan, both of which have been implicated in virulence and saprophytic fitness of the bacteria. Expression of *algD*, the first gene of the alginate biosynthetic operon, was shown to be induced at 28 °C and was significantly lower at 18 °C (Peñaloza-Vázquez et al. 1997). Alginate biosynthesis in *P. syringae* is controlled by the alternate sigma factor AlgT, whose expression is heat-shock inducible (Keith and Bender 1999), suggesting that alginate production might function in protecting the cell from heat stress. As described for the opportunistic human pathogen *P. aeruginosa*, an *algT* mutant of *P. syringae* was impaired in heat stress survival (Keith and Bender 1999) and so was a mutant defective in *mucD*, which encodes a periplasmic serine protease and is thought to be co-transcribed with *algT* (our unpublished observations). In contrast, a much more pronounced secretion of levansucrase, which is required for the synthesis of the second EPS of *P. syringae*, levan, was observed at 18 °C as compared to 28 °C (Li and Ullrich 2001; our unpublished observations). Since transcription of levansucrase genes was not affected by temperature, it is intriguing to speculate that the machinery required for levansucrase secretion underlies thermo-control. For *E. amylovora*, production of both major EPSs, amylovoran and levan, was shown to be induced by lower temperatures due to thermoregulation at the level of gene expression (Bereswill et al. 1997; Kelm et al. 1997).

Conclusions and outlook

Temperature responsiveness of gene expression occurs in all bacteria, which have developed divergent skills to adapt to their respective habitats. Research of the past decade has revealed solid evidence for thermoregulation of virulence in plant pathogens. For most of the studied

systems, temperature sensors remain to be identified – and for none of these systems is the sensory mechanism satisfactorily understood. Temperature is just one of many environmental parameters influencing gene expression or protein secretion. Consequently, the sensory proteins or principles might differ from system to system. Since the temperature ranges of interest differ considerably from those relevant to pathogens of mammals, one can predict that the molecular basis for thermoregulation in plant-associated bacteria must be novel. Two common principles for pathogens of mammals and those of plants emerge regarding temperature control of virulence expression: (1) rarely do heat or cold shock proteins play a role for the outcome of virulence; and (2) two-component regulatory cascades involved in thermoresponsive gene expression can be found in both groups of pathogens. In two systems from plant pathogens, the VirA/G system of *A. tumefaciens* and the CorS/R/P system of *P. syringae*, the sensor kinases might function as molecular thermometers being anchored in the cell's inner membrane. While for VirA periplasmic domains interacting with chemical signals have been determined, no such chemical signals have been identified to interact with CorS. However, a common feature for both could be that the degree of saturation of the fatty acid chains of membrane phospholipids may influence protein conformation. It is well established that unsaturated fatty acids are incorporated into the bacterial membrane in response to temperature downshifts. Therefore, future studies should focus on the interaction between the degree of saturation of the membrane fatty acids and the conformation and activity of the sensory protein(s). Moreover, global strategies should be employed to potentially identify regulatory networks and hierarchies for thermoregulation. Rohde et al. (1999) and Ullrich et al. (2000) have employed the techniques of two-dimensional protein profiling and promoter-trapping to identify novel thermoresponsive genetic loci in *P. syringae*. In the future, the DNA array technique should be the method of choice to continue with these investigations.

Each individual plant-microbe interaction has evolved a distinct and often unique combination of virulence and fitness determinants that may or may not underlie thermoregulation. Based on epidemiological data, perhaps most of these host-pathogen interactions depend on temperature in one way or the other (Ellingboe 2001). However, only a few well-investigated model systems for thermoresponsiveness in plant pathogens have been reported thus far; these have been summarized here. The thermoresponsiveness of other pathogenic plant-microbe interactions remain to be studied at the molecular level. This current mini-review was also designed to encourage other investigators to look at their model systems with respect to temperature effects.

References

Banta, LM, Bohne J, Lovejoy SD, Dostal K (1998) Stability of the *Agrobacterium tumefaciens* VirB10 protein is modulated by growth temperature and periplasmic osmoadaptation. J Bacteriol 180:6597–6606

Bender C, Palmer D, Peñaloza-Vázquez A, Rangaswamy V, Ullrich M (1996) Biosynthesis of coronatine, a thermoregulated phytotoxin produced by the phytopathogen *Pseudomonas syringae*. Arch Microbiol 166:71–75

Bereswill S, Jock S, Aldridge P, Janse JD, Geider K (1997) Molecular characterization of natural *Erwinia amylovora* strains deficient in levan synthesis. Physiol Mol Plant Pathol 51:215–225

Budde IP, Rohde BH, Bender CL, Ullrich MS (1998) Growth phase and temperature influence promoter activity, transcript abundance, and protein stability during biosynthesis of the *Pseudomonas syringae* phytotoxin coronatine. J Bacteriol 180: 1360–1367

Ellingboe AH (2001) Plant-pathogen interactions: genetic and comparative analyses. Eur J Plant Pathol 107:79–84

Hayward AC (1991) Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu Rev Phytopathol 29:65–87

Hugouvieux-Cotte-Pattat N, Dominguez H, Robert-Baudouy J (1992) Environmental conditions affect transcription of the pectinase genes of *Erwinia chrysanthemi* 3937. J Bacteriol 174: 7807–7818

Hugouvieux-Cotte-Pattat N, Condemine G, Nasser W, Reverchon S (1996) Regulation of pectinolysis in *Erwinia chrysanthemi*. Annu Rev Microbiol 50:213–257

Hurme R, Rhen M (1998) Temperature sensing in bacterial gene regulation – what it all boils down to. Mol Microbiol 30:1–6

Jin S, Song YN, Deng WY, Gordon MP, Nester EW (1993) The regulatory VirA protein of *Agrobacterium tumefaciens* does not function at elevated temperatures. J Bacteriol 175:6830–6835

Kado, CE (1998) Agrobacterium-mediated horizontal gene transfer. Genet Eng 20:1–24

Keith LM, Bender CL (1999) AlgT (?[22]) controls alginate production and tolerance to environmental stress in *Pseudomonas syringae*. J Bacteriol 181:7176–7184

Kelm O, Kiecker C, Geider K, Bernard F (1997) Interaction of the regulator proteins RcsA and RcsB with the promoter of the operon for amylovoran biosynthesis in *Erwinia amylovora*. Mol Gen Genet 256:72–83

Konkel ME, Tilly K (2000) Temperature-regulated expression of bacterial virulence genes. Microbes Infect 2:157–166

Lai EM, Kado CI (1998) Processed VirB2 is the major subunit of the promiscuous pilus of *Agrobacterium tumefaciens*. J Bacteriol 180:2711–2717

Lanham PG, McIlravey KI, Perombelon MCM (1991) Production of the cell wall dissolving enzymes by *Erwinia carotovora* subsp. *atroseptica* in vitro at 27°C and 30.5°C. J Appl Bacteriol 70:20–24

Li H, Ullrich MS (2001) Characterization and mutational analysis of three allelic *lsc* genes encoding levansucrase in *Pseudomonas syringae*. J Bacteriol 183:3282–3292

Mekalanos JJ (1992) Environmental signals controlling expression of virulence determinants in bacteria. J Bacteriol 174:1–7

Nemececk-Marshall M, LaDuca R, Fall R (1993) High-level expression of ice nuclei in a *Pseudomonas syringae* strain is induced by nutrient limitation and low temperature. J Bacteriol 175:4062–4070

Palmer DA, Bender CL (1993) Effects of environmental and nutritional factors on production of the polyketide phytotoxin coronatine by *Pseudomonas syringae* pv. *glycinea*. Appl Environ Microbiol 59:1619–1626

Peñaloza-Vázquez A, Bender CL (1998) Characterization of CorR, a transcriptional activator which is required for biosynthesis of the phytotoxin coronatine. J Bacteriol 180:6252–6259

Peñaloza-Vázquez A, Kidambi SP, Chakrabarty AM, Bender CL (1997) Characterization of the alginate biosynthetic gene cluster in *Pseudomonas syringae* pv. *syringae*. J Bacteriol 179: 4464–4472

Phadtare S, Alsina J, Inouye M (1999) Cold-shock response and cold-shock proteins. Curr Opin Microbiol 2:175–180

Rangaswamy V, Bender CL (2000) Phosphorylation of CorS and CorR, regulatory proteins that modulate production of the phytotoxin coronatine in *Pseudomonas syringae*. FEMS Microbiol Lett 193:13–18

Rohde BH, Schmid R, Ullrich MS (1999) Thermoregulated expression and characterization of an NAD(P)H-dependent 2-cyclohexen-1-one reductase in the plant pathogenic bacterium *Pseudomonas syringae* pv. *glycinea*. J Bacteriol 181:814–822

Rowley KB, Clements DE, Mandel M, Humphreys T, Patil SS (1993) Multiple copies of a DNA sequence from *Pseudomonas syringae* pv. *phaseolicola* abolish thermoregulation of phaseolotoxin production. Mol Microbiol 8:625–635

Rowley KB, Xu R, Patil SS (2000) Molecular analysis of thermoregulation of phaseolotoxin-resistant ornithine carbamoyltransferase (*argK*) from *Pseudomonas syringae* pv. *phaseolicola*. Mol Plant-Microbe Interact 13:1071–1080

Ullrich M, Peñaloza-Vázquez A, Bailey AM, Bender CL (1995) A modified two-component regulatory system is involved in temperature-dependent biosynthesis of the *Pseudomonas syringae* phytotoxin coronatine. J Bacteriol 157:6160–6169

Ullrich MS, Schergaut M, Boch J, Ullrich B (2000) Temperature-responsive genetic loci in the plant pathogen *Pseudomonas syringae*. Microbiol 146:2457–2468

Van Dijk K, Fouts DE, Rehm AH, Hill RA, Collmer A, Alfano JR (1999) The Avr (effector) proteins HrmA (HopPsyA) and AvrPto are secreted in culture from *Pseudomonas syringae* pathovars via the Hrp (type III) protein secretion system in a temperature- and pH-sensitive manner. J Bacteriol 181:4790–4797

Wang L, Bender CL, Ullrich MS (1999) The transcriptional activator CorR is involved in biosynthesis of the phytotoxin coronatine and binds to the *cmaABT* promoter region in a temperature-dependent manner. Mol Gen Genet 262:250–260

Wei ZM, Sneath BJ, Beer SV (1992) Expression of *Erwinia amylovora hrp* genes in response to environmental stimuli. J Bacteriol 174:1875–1882

Yura T, Nakahigashi K (1999) Regulation of the heat-shock response. Curr Opin Microbiol 2:153–158