Genetics of toxin production and resistance in phytopathogenic bacteria

D. K. Willis*†, T. M. Barta* and T. G. Kinscherf*

† *Agricultural Research Service, USDA and* * *Department of Plant Pathology, University of Wisconsin, Madison (Wisconsin 53706, USA)*

Abstract. Genes for phytotoxin production have been identified and cloned from several phytopathogenic pseudomonads. These genes comprise physically linked clusters that have been located both on the chromosome and on endogenous plasmids. Contained within these genetic regions are resistance genes specific to those toxins that have a bactericidal component to their activity. DNA sequences required for toxin production are often conserved among bacteria with divergent host specificities, suggesting the ability of toxin genes to be transferred between bacteria. Toxins are usually modulators of plant pathogenicity, their production causing a significant increase in disease severity. In one case, however, toxin production appears to be a major contributor to the basic pathogenicity of a plant pathogenic bacterium.

Key words. Tabtoxin; phaseolotoxin; coronatine; syringotoxin; syringomycin; resistance; *Pseudomonas syringae*, pv. *coronafaciens*, pv. *phaseolicola*, pv. *tabaci* (*angulata*), pv. *striafaciens*, pv. *tomato*.

Introduction

Toxins represent the best biochemically described class of compounds that are known to be involved in the pathogenicity of bacteria on plants. The chemical structure of toxins, the nature of intermediates in their biosynthesis, and their mode of action in plant disease is known in many cases. Production of a particular toxin is often distributed among bacteria that differ in host specificities. This wide distribution raises the possibility of the physical movement of toxin biosynthetic and resistance genes, through transposition, natural transformation, or plasmid transfer, resulting in symptomatic flexibility by the recipient bacteria. It is only recently that significant progress has been made on the genetic analysis of toxin production, particularly at the molecular level. We present a brief summary of the current state of our knowledge of the genetics of toxin production and resistance in plant pathogenic bacteria. We have chosen not to discuss the well-characterized and recently reviewed plant growth altering bacteria *Agrobacterium tumefaciens* and *Pseudomonas syringae* pv. *savastanoi*[44, 54]. This review will focus on the recent advances made in the genetic analysis of phaseolotoxin production and resistance in *P. syringae* pv. *phaseolicola*, the production of coronatine by *P. syringae* pv. *tomato*, syringomycin and syringotoxin production by *P. syringae* pv. *syringae*, and tabtoxin production by isolates and pathovars of *P. syringae*.

Phaseolotoxin production and resistance

P. syringae pv. *phaseolicola*, the causal agent of halo blight of bean (*Phaseolus vulgaris*), produces a chlorosis-inducing phytotoxin designated as phaseolotoxin. This toxin is a tripeptide whose structure has been determined to be N^{δ}-(N'-sulpho-diaminophosphinyl)-L-ornithylalanylhomoarginine[34, 41]. The toxin, as produced by bacteria, is thought to be a relatively inactive precursor, with maturation occurring in planta as a result of processing by plant peptidases to yield the active moiety N^{δ}-(N'-sulpho-diaminophosphinyl)-L-ornithine, abbreviated as PSorn or, more recently, octicidine[34, 37, 41]. Phaseolotoxin is a specific inhibitor of ornithine carbamoyltransferase (OCTase) in both plants and bacteria[14, 48–50, 63]. Production of phaseolotoxin is temperature regulated with optimal production occurring between 18 and 20 °C, and no detectable production at 30 °C[18, 61]. The role of phaseolotoxin as a virulence factor, which is responsible for an increase in disease severity, has been established by the analysis of naturally-occurring or mutagen-induced phaseolotoxin-deficient (Tox^-) mutants[18, 47, 51, 61]. All such phaseolotoxin-deficient mutant strains are still able to cause lesions on the leaves of bean but lack the characteristic chlorotic halo typical of the disease and are unable to spread systemically through the host plant.

Recently, a chromosomal gene cluster required for phaseolotoxin production has been identified through transposon mutagenesis and cloned from *P. syringae* pv. *phaseolicola*[51]. In a screen of 5200 kanamycin-resistant (Kan^r) mutants, six independent Tn*5* insertions were isolated that gave rise to a phaseolotoxin-reduced phenotype. All six of these transposon mutations were found to be linked on a single 28 kb *Kpn*I restriction fragment. The mutants were restored to toxin production by the introduction of wild-type DNA sequences homologous to the region containing the Tn*5* insertions. The genes involved are apparently unique to phaseolotoxin-producing *P. syringae* pv. *phaseolicola* strains, since probes from the region did not hybridize to total DNA isolated from closely-related *P. syringae* that do not produce the toxin. It is interesting to note that none of the Tn*5* insertions completely eliminated toxin production, implying that either the major biosynthetic genes in the toxin pathway were not disrupted or that the mutants may be producing an intermediate in phaseolotoxin synthesis that has an inhibitory effect.

In bacteria, phaseolotoxin is taken up by the oligopeptide permease system. Previous work has demonstrated

that resistance to phaseolotoxin can be acquired by *Salmonella typhimurium* and *Escherichia coli* through the loss of this oligopeptide permease system, thereby eliminating the ability to transport the toxin to the intracellular space[62]. Apparently, this is not the method used by phaseolotoxin-producing bacteria for self-protection. To prevent autotoxicity, *P. syringae* pv. *phaseolicola* produces a phaseolotoxin-insensitive OCTase[13,63,67] under temperature conditions (18 °C) that favor phaseolotoxin production[63]. A second OCTase is expressed at 30 °C, a temperature at which phaseolotoxin is not produced by the bacteria[63]. The genes coding for both the resistant and sensitive OCTase enzymes have been isolated and are functionally expressed in *E. coli*[52]. Both of the *P. syringae* pv. *phaseolicola* OCTase genes were found to restore OCTase-deficient strains of *E. coli* and *P. aeruginosa* to growth without arginine supplements. Interestingly, expression of the cloned toxin-resistant OCTase was not temperature regulated in *E. coli*. It is unclear why the *P. syringae* pv. *phaseolicola* produces the second, toxin-sensitive enzyme when the resistant OCTase appears completely adequate for growth. Finally, hybridization and complementation studies indicate that the gene(s) encoding the toxin-insensitive enzyme are part of the gene cluster involved in phaseolotoxin production described above[51,52]. Such linkage is not at all uncommon, or illogical, for antibiotic and toxin resistance[33].

Coronatine production

Isolates of *P. syringae* pvs. *tomato*, *atropurpurea*, *glycinea*, and *morsprunorum*, which cause disease on tomato, ryegrass, soybean and *Prunus* spp., respectively, produce coronatine[35,38,39,45]. This toxin consists of a polyketide component (coronafacic acid) joined to a cyclopropane structure derived from leucine[23,36,46]. Although the mode of action is not yet known, the toxin is associated with stunting, chlorosis, and hypertrophy of plant tissue[16,56].

Bender et al.[3] investigated 38 coronatine-deficient (Cor^-) mutants that were identified following Tn*5* mutagenesis of *P. syringae* pv. *tomato* (strain PT23.2). Five mutants contained Tn*5* insertions in the 101 kb indigenous plasmid pPT23A. The remainder of the mutants had either lost pPT23A or were missing parts of this plasmid. Furthermore, this plasmid conferred coronatine production to a strain of *P. syringae* pv. *syringae* which does not naturally produce this toxin. Other coronatine-producing strains of *P. syringae* pv. *glycinea* and *P. syringae* pv. *atropurpurea* have plasmids that hybridized to the regions of pPT23A involved in coronatine production[2]. Site-directed mutagenesis of *P. syringae* pv. *glycinea* with Tn*5*-mutated coronatine sequences from *P. syringae* pv. *tomato* resulted in reduced coronatine production, suggesting that there may be conservation of coronatine genes between these pathovars[2]. Genes required for coronatine production also have been associated with plasmid DNA in *P. syringae* pv. *atropurpurea*[57,58]. However, when another laboratory generated Cor^- Tn*5* mutants of *P. syringae* pv. *tomato* (strain DC3000), four of five Tn*5* insertions were linked on a 19 kb region of chromosomal DNA[40]. Thus, it would appear that while the plasmid location of coronatine biosynthetic genes may facilitate their dispersal, an extrachromosomal location is not required for their expression.

Mutants of *P. syringae* pv. *tomato* with reduced or no coronatine production still produced lesions on tomato plants, but the lesions were reduced in size[4]. Also, population densities of these mutants in tomato leaves were significantly lower than the wild-type strain[4]. These data suggest that, like phaseolotoxin and as will be seen with syringomycin, coronatine appears to be a virulence factor, i.e. it is not required for lesion formation.

Syringomycin and syringotoxin production

The majority of *P. syringae* pv. *syringae* strains produce one of two plant necrosis-inducing toxins that have been implicated as virulence factors in disease[20,65,74]. *P. syringae* pv. *syringae* isolates from a wide variety of hosts produce syringomycin (SR), while syringotoxin (ST) production is associated with strains isolated from citrus[17,21]. The disruption of physiological functions within the plant plasma membrane has been implicated as the mode of action of syringomycin and isolated toxin causes necrosis when infiltrated into plant tissue[5,6,19,76]. Both SR and ST inhibit the growth of fungi such as *Geotrichum candidum* and *Rhodotorula pilimanae* and this property provides an useful bioassay for toxin production[20,76].

Tn*5* mutagenesis of the syringomycin-producing *P. syringae* pv. *syringae* (strain B301D), a pathogen of deciduous fruit trees, has led to the identification of two genetic loci, designated as *syrA* and *syrB*, that are required for SR production in vitro[74,75]. These loci have been cloned and the *syrA* coding region is estimated to be 2.3–2.8 kb in length while *syrB* encompasses an approximately 3 kb region[75]. A mutation in either of theses loci result in the loss of two large molecular weight proteins designated SR4 (~350 kDa) and SR5 (~130 kDa) as determined by SDS-PAGE electrophoresis[75]. The relationship between *syrA* or *syrB* and the SR4 and SR5 proteins remains unclear, since neither locus contains enough capacity to encode proteins of this size. Recently, an additional locus, designated as *syrD*, has been identified that is physically linked to *syrB*. Transposon insertions in *syrD* prevent transcription of a *syrB::lacZ* fusion, suggesting a regulatory relationship between these two loci[53].

Mutations in the different genetic loci required for syringomycin production have distinct and different effects on plant pathogenicity[74]. Tn*5* insertions in *syrA* eliminate both SR production in vitro and pathogenicity in

planta. On the other hand, *syrB* mutants are SR^- in vitro but still pathogenic on immature cherry fruit, although the disease index is significantly reduced. The *syrB* locus is hypothesized to be directly involved in the synthesis of SR[75]. In contrast to the findings of Xu and Gross, SR is apparently not a virulence factor in brown spot disease of bean caused by *P. syringae* pv. *syringae* (strain B728a). We have isolated 11 Tn*5* mutants that are SR^- in vitro but retained full pathogenicity, suggesting that SR is not required for lesion formation on bean[22] (E. M. Hrabak and D. K. Willis, unpublished results).

Morgan and Chatterjee have identified a 32 kb genomic region that is required for syringotoxin production by citrus isolates of *P. syringae*[42,43]. Twelve independent Tn*5* insertions that eliminated the production of ST were found to be linked and all were contained within this region. Two large proteins, designated as ST1 (470 kDa) and ST2 (435 kDa), are apparently encoded by this region. This conclusion is supported by a significant correlation between the position of transposon insertions within the region and the appearance of smaller, presumably truncated, proteins in SDS-PAGE gels[43]. Recently, two overlapping cosmid clones have been isolated that restore all twelve of the Tn*5* generated ST^- insertions[9]. To date, the effect of the loss of ST production on plant pathogenicity has not been reported.

Tabtoxin production and resistance

Tabtoxin is a dipeptide toxin precursor produced by several pathovars and isolates of *Pseudomonas syringae*. These bacteria include *P. syringae* pv. *tabaci*, the causative agent of wildfire of tobacco[10,24]; *P. syringae* isolate BR2, wildfire of bean; *P. syringae* isolate 0152, wildfire of soybean[55]; *P. syringae* pv. *coronafaciens*, haloblight of oats[12]; and *P. syringae* pv. *garcae*, bacterial scorch of coffee (R. D. Durbin, personal communication). While tabtoxin is synthesized in a biologically-inactive form, it is readily cleaved by aminopeptidases present in either the bacteria or the plant, yielding threonine (or serine for 2-serine-tabtoxin) and the active moiety tabtoxinine-β-lactam[11,30,72]. Tabtoxinine-β-lactam (TβL) and tabtoxin are structurally related to the β-lactam antibiotics which include penicillins and cephalosporins[11,28,64,66,72]. In contrast to penicillin-like β-lactam antibiotics that are inhibitors of bacterial cell wall synthesis, TβL specifically affects glutamine synthetase (GS)[60,69,70]. The toxin has been reported to bind irreversibly to the enzyme[29,69], inactivating it in a manner similar to another GS inhibitor, methionine sulfoximine (MSO)[31]. Both MSO and TβL are general GS inhibitors, having been shown to affect the enzyme in bacteria[69], plants[70], and fungi (R. D. Durbin, personal communication). The accumulation of ammonia due to GS inhibition is thought to be the major factor associated with the in planta chlorosis produced by this toxin[71].

Tabtoxin-producing bacteria are resistant to TβL and several mechanisms have been proposed to account for this resistance. Glutamine synthetase isolated from *P. syringae* pv. *tabaci* is sensitive to TβL and there is no evidence for the synthesis of a resistant GS in this bacterium[69]. However, adenylylation of the resident GS is increased by the presence of TβL, and this modification provides significant protection from inhibition in vitro[26]. Another proposed mechanism for resistance involves the production of a β-lactamase activity that was detected in extracts from Tox^+ TβL-resistant (Tox^r) but not Tox^- TβL-sensitive (Tox^s) *P. syringae* pv. *tabaci*[27]. Lastly, a tabtoxin-specific transacetylase activity has recently been described to account for self-protection by *P. syringae* pv. *tabaci*.[1].

Two novel approaches to obtaining tabtoxin resistance have been described that use MSO as a selective agent. Carlson[8] selected MSO-resistant (MSO^r) calli from a population of ethyl methanesulfonate-mutagenized haploid tobacco tissue-culture cells. Plants regenerated from MSO^r callus did not exhibit the chlorotic halos normally associated with *P. syringae* pv. *tabaci* infection. Instead, the reaction shown by this bacterium on MSO^r tobacco were similar to the symptoms produced by the inoculation of *P. syringae* pv. *tabaci* (*angulata*). Leaf tissue from the MSO^r tobacco plants were found to have elevated levels of methionine, but the relationship between the increased level of this amino acid and MSO resistance was not established. Overproduction of GS, through the placement of the glutamine synthetase gene on a multicopy plasmid, leads to an increase in MSO and tabtoxin resistance in the yeast *Saccharomyces cerevisiae*[32]. The authors also found that three additional DNA sequences, unrelated to the GS gene or each other, increased resistance to MSO and tabtoxin when on a multicopy plasmid. The latter three DNA sequences conferred resistance to MSO by blocking intracellular transport of the drug.

Angular leafspot of tobacco is caused by a group of bacteria taxonomically related to *P. syringae* pv. *tabaci* that was previously designated as *Pseudomonas angulata*. Wildfire and angular leafspot organisms are often isolated from the same diseased field and cannot be distinguished by physiological, morphological, or serological characteristics[7]. The only well characterized difference between these pathogens is in the ability of wildfire isolates to produce tabtoxin, with the resultant plant symptoms caused by the toxin. Both *P. syringae* pv. *tabaci* (*angulata*) and spontaneous Tox^- mutants of *P. syringae* pv. *tabaci* are sensitive to TβL, indicating a possible functional linkage between tabtoxin synthesis and resistance genes. A similar dimorphic relationship exists between Tox^+ isolates of *P. syringae* pv. *coronafaciens*, the causal agent of haloblight of oats, and Tox^- variants known as *P. syringae* pv. *striafaciens*, the organism responsible for

bacterial stripe of oats. Except for the presence or absence of tabtoxin production and resistance, these two pathovars are virtually identical[59,68].

Our laboratory has been investigating the genetic control of tabtoxin production and resistance by analyzing Tox^- mutants of *P. syringae* isolate BR2, a causal agent of wildfire disease of bean (*Phaseolus vulgaris*)[25,73]. This isolate is distinct from *P. syringae* pv. *tabaci* in that it is pathogenic on bean but not on tobacco[55] (T. M. Barta and D. K. Willis, unpublished data). 7,000 kanamycin-resistant transconjugants of BR2 that were screened by bioassay (15) and 23 Tox^- mutants were identified. Of these, 20 mutants were TβL-sensitive (Tox^s) while three retained toxin resistance (Tox^r). The three Tox^- Tox^r mutants have Tn*5* insertions that are linked on a single 22 kb *Eco*RI fragment, whereas, the transposon insertions within the Tox^- Tox^s bacteria appear to be unlinked. Use of the cloned Tn*5* insertion region from one of the Tox^- Tox^r mutants as a probe in hybridization analyses revealed that the corresponding region was deleted from all of the Tox^- Tox^s bacteria. At least 8.5 kb of this region was found to be conserved, with respect to homology and length of restriction fragments, in all tabtoxin-producing *P. syringae* pathovars examined. This conserved region was not present in any of the naturally-occurring Tox^- isolates, such as *P. syringae* pv. *tabaci* (*angulata*) and *P. syringae* pv. *striafaciens*, that we examined. Laboratory generated spontaneous Tox^- derivatives of *P. syringae* pv. *tabaci* were also found to be deleted for this region. These deletion events appeared to involve a common mechanism, in that the excision in all cases was precise to at least 200 bp. The exact precision of deletion has not been determined and will require sequence analysis of junction fragments. The cloned, wild-type 22 kb *Eco*RI fragment restored all of the Tox^- Tox^r Tn*5* mutants to Tox^+. The same clone was unable to restore deletion mutants of BR2 indicating that additional DNA sequence is required for toxin production. Cosmids containing larger DNA inserts corresponding to the region have been isolated, and these appear to restore the Tox^- Tox^s deletion mutants of BR2. Use of these cosmids as hybridization probes indicated that the total extent of the DNA deleted in the spontaneous BR2 Tox^- Tox^s mutants is on the order of 25–30 kb. Resistance to TβL was linked to these cosmids and was at least partially expressed in *E. coli* (T. G. Kinscherf and D. K. Willis, unpublished data).

In a similar mutational analysis of *P. syringae* pv. *coronafaciens* (strain Pc27), seven Tn*5*-generated Tox^- strains were isolated after screening 3400 Kan^r mutants by bioassay. All seven of these mutants retained their resistance to TβL. The Tn*5* insertions in the seven mutants were linked on a 17 kb *Kpn*I fragment. Strikingly, this region is completely distinct from the deleted region described above, which appears to be intact in the Pc27 mutants. It would appear that in *P. syringae* pv. *coronafaciens* additional gene regions play a role in the production of tabtoxin (T. M. Barta and D. K. Willis, unpublished data).

Unlike the other toxins discussed in this review, loss of tabtoxin production has a varied impact on the pathogenicity of the organism. Toxin-deficient mutants of either *P. syringae* pv. *tabaci* or *P. syringae* pv. *coronafaciens* retained the ability to cause disease lesions, albeit without chlorosis, identical to the symptoms produced by their respective naturally-occurring Tox^- derivatives, *P. syringae* pv. *tabaci* (*angulata*) and *P. syringae* pv. *striafaciens* (T. M. Barta, T. G. Kinscherf and D. K. Willis, unpublished data). In contrast, Tox^- mutants of *P. syringae* BR2, whether transposon-generated or spontaneous deletions, did not produce detectable symptoms on bean[25].

Conclusions

Researchers are just beginning to dissect the genes and gene products that are required for bacterial phytotoxin production. Even though our accumulated knowledge is relatively primitive, several important findings are emerging. Genes required for toxin production by the phytopathogenic pseudomonads, as has been found in the production of bacterially encoded antibiotics, are clustered and linked to toxin resistance loci. It is expected that further analysis of these gene clusters will reveal coordinate regulation between toxin synthesis and the expression of resistance genes, an obvious requirement to avoid autotoxicity. The observation that DNA sequences required for toxin production are conserved among bacteria with distinct host specificities suggests that these genes have moved in the recent past, supporting the hypothesis that toxin synthetic genes are sometimes contained within mobile genetic elements. The finding that tabtoxin is the major pathogenicity factor of *P. syringae* strain BR2 suggests that, is some cases at least, nonpathogenic bacterial epiphytes can become plant pathogens upon acquiring the capability to produce a phytotoxin. The potential movement of toxin genes into a wide variety of phytopathogenic bacteria accompanied by a coincident increase in virulence does not bode well for agriculture. The proximity provided by the methods of modern intensive agriculture provide a 'melting pot' environment that may become a proving ground for different combinations of bacteria and toxins. It is possible that the acquisition of a single gene cluster would be enough to create a novel plant pathogen.

However, increasing our understanding of the biological processes involved in plant pathogenesis and the cloning of phytotoxin resistance genes also opens up new possibilities for the development of disease-resistant plants. For example, transgenic tobacco plants expressing resistance to TβL have been constructed and, in preliminary experiments, have been shown to exhibit a significant reduction in disease symptoms when inoculated with *P. syringae* pv. *tabaci*[1]. Further investigations into the

biosynthesis of phytotoxins and the resistance mechanisms employed by the bacteria that produce them offers the possibility of controlling bacterial diseases through innovative means.

Addendum in proof

During the interval between the submission of the revised manuscript and our receipt of proof pages, several papers have been published that pertain to the genetic analyses of toxin production discussed in this review. With respect to coronatine production, Bender et al. (Bender, C. L., Young, S. A., and Mitchell, R. E., Conservation of plasmid DNA sequences in coronatine-producing pathovars of *Pseudomonas syringae*. Appl. envir. Microbiol. *57* (1991) 993–999.) have described the physical and functional conservation of a 30 kb region of plasmid DNA among plasmids within the *P. syringae* pathovars *atropurea, glycinea,* and *morsprunorum.* In other work, the genes required for coronatine production in *P. syringae* pv. *tomato* have been localized to a 30 kb region of the chromosome using saturation transposon mutagenesis with Tn*3*-Spice and Tn*phoA* (Ma, S.-W., Morris, V. L., and Cuppels, D. A., Characterization of a DNA region required for production of the phytotoxin coronatine by *Pseudomonas syringae* pv. *tomato*. Molec. Plant-Micro. Interact. *4* (1991) 69–74.). Six complementation groups were identified but, based on Tn*phoA* insertions, none of the affected loci encode secreted proteins. From analysis of Tn*3*-Spice fusions to loci within one of the complementation groups, bacteria grown on tomato plants exhibited a 370-fold increase in gene expression over in vitro grown bacteria.

A difference in the expression of the *syrB* gene has recently been noted between two strains of *P. syringae* pv. *syringae* (Mo, Y.-Y., and Gross, D. C., Expression in vitro and during plant pathogenesis of the *syrB* gene required for syringomycin production by *Pseudomonas syringae* pv. *syringae*. Molec. Plant-Micro. Interact. *4* (1991) 28–36.). In strain B301D, syringomycin production and *syrB* expression (as measured by a *lacZ* reporter fusion) are constitutive. However, strain B3AR132 produces SR on minimal medium only in the presence of plant extracts. Consistent with this observation, a *syrB*::*lacZ* fusion introduced into B3AR132 only expresses β-galactosidase in the presence of plant extracts. This result suggests that plant molecules are important for the expression of SR in at least some *P. syringae* pv. *syringae* strains.

We have recently isolated a cosmid clone from a *P. syringae* BR2 genomic library that encodes all of the information necessary for the production of and resistance to tabtoxin (Kinscherf, T. G., Coleman, R. H., Barta, T. M., and Willis, D. K., Cloning and expression of tabtoxin biosynthesis and resistance from *Pseudomonas syringae*. J. Bact. *173* (1991) 4124–4132.). The plasmid pRTBL823, when introduced into a tabtoxin-naive *P. syringae* strain such as Cit7, leads to the production of tabtoxin by this strain. We have localized the tabtoxin biosynthetic genes to an approximately 26 kb region of the chromosome. We have also cloned the tabtoxin biosynthetic region from *P. syringae* pv. *coronafaciens.*

1 Anzai, H., Yoneyama, K., and Yamaguchi, I., Transgenic tobacco resistant to a bacterial disease by the detoxification of a pathogenic toxin. Molec. gen. Genet. *219* (1989) 492–494.
2 Bender, C. L., and Malvick, D. K., Coronatine-producing strains of *Pseudomonas syringae* pathovars *tomato, glycinea,* and *atropurpurea* share related plasmid DNA sequences. Phytopathology *78* (1988) 1527.
3 Bender, C. L., Malvick, D. K., and Mitchell, R. E., Plasmid-mediated production of the phytotoxin coronatine in *Pseudomonas syringae* pv. *tomato*. J. Bact. *171* (1989) 807–812.
4 Bender, C. L., Stone, H. E., Sims, J. J., and Cooksey, D. A., Reduced pathogen fitness of *Pseudomonas syringae* pv. *tomato* Tn*5* mutants defective in coronatine production. Physiol. molec. Plant Path. *30* (1987) 273–283.
5 Bidwai, A. P., and Takemoto, J. Y., Bacterial phytotoxin, syringomycin, induces a protein kinase-mediated phosphorylation of red beet plasma membrane polypeptides. Proc. natl Acad. Sci. USA *84* (1987) 6755–6759.
6 Bidwai, A. P., Zhang, L., Bachmann, R. C., and Takemoto, J. Y., Mechanism of action of *Pseudomonas syringae* phytotoxin, syringomycin. Stimulation of red beet plasma membrane ATPase activity. Plant Physiol. *83* (1987) 39–43.
7 Braun, A. C., A comparative study of *Bacterium tabacum* Wolf and Foster and *Bacterium angulatum* Fromme and Murray. Phytopathology *27* (1937) 283–304.
8 Carlson, P. S., Methionine sulfoximine-resistant mutants of tobacco. Science *180* (1973) 1366–1368.
9 Chatterjee, A. K., Somylai, G., and Nordeen, R. O., Molecular cloning and expression of syringotoxin (*syt*) genes of *Pseudomonas syringae* pv. *syringae*, in: Pseudomonas: Biotransformations, Pathogenesis, and Evolving Biotechnology, pp. 58–63. Eds S. Silver, A. N.-Chakrabarty, B. Inglewski and S. Kaplan. American Society for Microbiology, Washington, DC 1990.
10 Clayton, E. E., Toxin produced by *Bacterium tabacum* and its relation to host range. J. agric. Res. *48* (1934) 411–426.
11 Durbin, R. D., Uchytil, T. F., Steele, J. A., and Ribeiro, L. D., Tabtoxinine-β-lactam from *Pseudomonas tabaci*. Phytochemistry *17* (1978) 147–148.
12 Elliott, C., Halo blight of oats. J. agric. Res. *19* (1920) 139–172.
13 Ferguson, A. R., Johnson, J. S., and Mitchell, R. E., Resistance of *Pseudomonas syringae* pv. *phaseolicola* to its own toxin, phaseolotoxin. Fedn Eur. Microbiol. Soc. Lett. *7* (1980) 123–125.
14 Ferguson, A. R., and Johnston, J. S., Phaseolotoxin: chlorosis, ornithine accumulation and inhibition of ornithine carbamoyltransferase in different plants. Physiol. Plant Path. *16* (1980) 269–275.
15 Gasson, M. J., Indicator technique for antimetabolic toxin production by phytopathogenic species of *Pseudomonas*. Appl. envir. Microbiol. *39* (1980) 25–29.
16 Gnanamanickam, S. S., Starratt, A. N., and Ward, E. W. B., Coronatine production in vitro and in vivo and its relation to symptom development in bacterial blight of soybean. Can. J. Bot. *60* (1982) 645–650.
17 Gonzalez, C. F., DeVay, J. E., and Wakeman, R. J., Syringotoxin: a phytotoxin unique to citrus isolates of *Pseudomonas syringae*. Physiol. Plant Path. *18* (1981) 41–50.
18 Goss, R. W., The relationship of temperature to common and halo blight of beans. Phytopathology *30* (1940) 258–264.
19 Gross, D. C., and Cody, Y. S., Mechanisms of plant pathogenesis by *Pseudomonas* species. Can. J. Microbiol. *31* (1985) 403–410.
20 Gross, D. C., and DeVay, J. E., Population dynamics and pathogenesis of *Pseudomonas syringae* in maize and cowpea in relation to the in vitro production of syringomycin. Phytopathology *67* (1977) 475–483.
21 Gross, D. C., and DeVay, J. E., Production and purification of syringomycin, a phytotoxin produced by *Pseudomonas syringae*. Physiol. Plant Path. *11* (1977) 13–28.
22 Hrabak, E. M., Rich, J. J., Kennedy, C. J., and Willis, D. K., A locus required for lesion formation by *Pseudomonas syringae* pv. *syringae* on bean affects syringomycin production in vitro. Phytopathology *79* (1989) 1178 (abstract).

23 Ichihara, A., Shiraishi, K., Sato, H., Sakamura, S., Nishiyama, K., Sakai, R., Furasaki, A., and Matsumota, T., The structure of coronatine. J. Am. chem. Soc. *99* (1977) 636–637.

24 Johnson, J., and Murwin, H. F., Experiments on the control of wildfire of tobacco, in: Research Bulletin *62*, Agricultural Experiment Station of the University of Wisconsin, Madison 1925.

25 Kinscherf, T. G., Coleman, R. H., Barta, T. M., and Willis, D. K., Isolation and characterization of a chromosomal region involved in tabtoxin production in the bean pathogen *Pseudomonas syringae* BR2. J. Bact., in press.

26 Knight, T. J., Durbin, R. D., and Langston-Unkefer, P. J., Role of glutamine synthetase adenylylation in the self-protection of *Pseudomonas syringae* subsp. *'tabaci'* from its toxin, tabtoxinine-β-lactam. J. Bact. *166* (1986) 224–229.

27 Knight, T. J., Durbin, R. D., and Langston-Unkefer, P. J., Self-protection of *Pseudomonas syringae* pv. *'tabaci'* from its toxin, tabtoxinine-β-lactam. J. Bact. *169* (1987) 1954–1959.

28 Koster, W. H., Cimarusti, C. M., and Sykes, R. B., Monobactams, in: Chemistry and biology of β-lactam antibiotics, pp. 339–375. Eds R. B. Morin and M. Gorman. Academic Press, New York 1982.

29 Langston-Unkefer, P. J., Macy, P. A., and Durbin, R. D., Inactivation of glutamine synthetase by tabtoxin-β-lactam. Effects of substrates and pH. Plant Physiol. *76* (1984) 71–74.

30 Levi, C., and Durbin, R. D., The isolation and properties of a tabtoxin-hydrolysing aminopeptidase from the periplasm of *Pseudomonas syringae* pv. *syringae*. Physiol. molec. Plant path. *28* (1986) 345–352.

31 Manning, J. M., Moore, S., Rowe, W. B., and Meister, A., Identification of L-methionine *S*-sulfoximine as the diastereoisomer of L-methionine *SR*-sulfoximine that inhibits glutamine synthetase. Biochemistry *8* (1969) 2681–2685.

32 Marek, E. T., and Dickson, R. C., Cloning and characterization of *Saccharomyces cerevisiae* genes that confer L-methionine sulfoximine and tabtoxin resistance. J. Bact. *169* (1987) 2440–2448.

33 Martin, J. F., and Liras, P., Organization and expression of genes involved in the biosynthesis of antibiotics and other secondary metabolites. A. Rev. Microbiol. *43* (1989) 173–206.

34 Mitchell, R. E., Isolation and structure of a chlorosis inducing toxin of *Pseudomonas phaseolicola*. Phytochemistry *15* (1976) 1941–1947.

35 Mitchell, R. E., Coronatine production by some phytopathogenic pseudomonads. Physiol. Plant Path. *20* (1982) 83–89.

36 Mitchell, R. E., Coronatine biosynthesis: incorporation of L-[U-^{14}C]isoleucine and L-[U^{14}C]threonine into the 1-amido-1-carboxy-2-ethylcyclopropyl moiety. Phytochemistry *24* (1985) 247–249.

37 Mitchell, R. E., and Bielski, R. L., Involvement of phaseolotoxin in halo blight of beans: transport and conversion to functional toxin. Plant Physiol. *60* (1977) 723–729.

38 Mitchell, R. E., Hale, C. N., and Shanks, J. C., Production of different pathogenic symptoms and different toxins by strains of *Pseudomonas syringae* pv. *tomato* not distinguishable by gel-immunodiffusion assay. Physiol. Plant Path. *23* (1983) 315–322.

39 Mitchell, R. E., and Young, H., Identification of a chlorosis-inducing toxin of *Pseudomonas glycinea* as coronatine. Phytochemistry *17* (1978) 2028–2029.

40 Moore, R. A., Starratt, A. N., Ma, S.-W., Morris, V. L., and Cuppels, D. A., Identification of a chromosomal region required for biosynthesis of the phytotoxin coronatine by *Pseudomonas syringae* pv. *tomato*. Can. J. Microbiol. *35* (1989) 910–917.

41 Moore, R. E., Niemczura, W. P., Kwok, O. C. H., and Patil, S. S., Inhibitors of ornithine carbamoyltransferase from *P. syringae* pv. *phaseolicola*. Revised structure of phaseolotoxin. Tetrahedron Lett. *25* (1984) 3931–3934.

42 Morgan, M. K., and Chatterjee, A. K., Isolation and characterization of Tn*5* insertion mutants of *Pseudomonas syringae* pv. *syringae* altered in the production of the peptide syringotoxin. J. Bact. *164* (1985) 14–18.

43 Morgan, M. K., and Chatterjee, A. K., Genetic organization and regulation of proteins associated with the production of syringotoxin by *Pseudomonas syringae* pv. *syringae*. J. Bact. *170* (1988) 5689–5697.

44 Morris, R. M., Genes specifying auxin and cytokinin biosynthesis in phytopathogens. A. Rev. Plant Physiol. *37* (1986) 509–538.

45 Nishiyama, K., Sakai, R., Ezuka, A., Ichihara, A., Shiraishi, K., Ogasawara, M., Sato, H., and Sakamura, S., Phytotoxic effect of coronatine produced by *Pseudomonas coronafaciens* var. *atropurpurea* on leaves of Italian ryegrass. Ann. Phytopath. Soc. Japan *42* (1976) 613–614.

46 Parry, R. J., and Mafoti, R., Biosynthesis of coronatine, a novel polyketide. J. Am. chem. Soc. *108* (1986) 4681–4682.

47 Patil, S. S., Emmons, B., and Hayward, C., An ultraviolet-induced nontoxigenic mutant of *Pseudomonas phaseolicola* of altered pathogenicity. Phytopathology *64* (1974) 590–595.

48 Patil, S. S., Kolatukudy, P. E., and Dimond, A. E., Inhibition of ornithine carbamoyltransferase from bean plants by the oxin of *Pseudomonas phaseolicola*. Plant Physiol. *46* (1970) 752–753.

49 Patil, S. S., Tam, L. Q., and Sakai, W. S., Mode of action of the toxin from *Pseudomonas phaseolicola*. I. Toxin specificity, chlorosis, and ornithine accumulation. Plant Physiol. *49* (1972) 803–807.

50 Patil, S. S., Youngblood, P., Christiansen, P., and Moore, R. E., Phaseotoxin A: an antimetabolite from *Pseudomonas phaseolicola*. Biochem. biophys. Res. Commun. *69* (1976) 1019–1027.

51 Peet, R. C., Lindgren, P. B., Willis, D. K., and Panopoulos, N. J., Identification and cloning of genes involved in phaseolotoxin production by *Pseudomonas syringae* pv. *'phaseolicola'*. J. Bact. *166* (1986) 1096–1105.

52 Peet, R. C., and Panopoulos, N. J., Ornithine carbamoyltransferase genes and phaseolotoxin immunity in *Pseudomonas syringae* pv. *syringae*. EMBO J. *6* (1987) 3585–3591.

53 Quigley, N. B., Mo, Y.-Y., and Gross, D. C., Genetic and DNA sequence analysis of the interval between two divergently transcribed phytotoxin biosynthesis genes from *Pseudomonas syringae* pv. *syringae*. Phytopathology *79* (1989) 1178–1179.

54 Ream, W., *Agrobacterium tumefaciens* and interkingdom genetic exchange. A. Rev. Phytopath. *27* (1989) 583–618.

55 Ribeiro, R. de L. D., Hagedorn, D. J., Durbin, R. D., and Uchytil, T. F., Characterization of the bacterium inciting bean wildfire in Brazil. Phytopathology *69* (1979) 208–212.

56 Sakai, R., Nishiyama, K., Ichihara, A., Shiraishi, K., and Sakamura, S., The relation between bacterial toxic action and plant growth regulation, in: Recognition and Specificity in Plant Host-Parasite Interactions, pp. 165–179. Eds J. M. Daly and I. Uritani. University Press, Baltimore, MD 1979.

57 Sato, M., A plasmid involved in coronatine production by *Pseudomonas syringae* pv. *atropurpurea*. Proc. Fifth International Congress of Plant Pathology, Kyoto, Japan 1988, p. 12.

58 Sato, M., Nishiyama, K., and Shirata, A., Involvement of plasmid DNA in the productivity of coronatine by *Pseudomonas syringae* pv. *atropurpurea*. Ann. Phytopath. Soc. Japan *49* (1983) 522–528.

59 Schaad, N. W., and Cunfer, B. M., Synonymy of *Pseudomonas coronafaciens*, *Pseudomonas coronafaciens* pathovar *zeae*, *Pseudomonas coronafaciens* subsp. *atropurpurea*, and *Pseudomonas striafaciens*. Int. J. syst. Bact. *29* (1979) 213–221.

60 Sinden, S. L., and Durbin, R. D., Glutamine synthetase inhibition: possible mode of action of wildfire toxin from *Pseudomonas tabaci*. Nature *229* (1968) 379–380.

61 Staskawicz, B. J., and Panopoulos, N. J., A rapid and sensitive microbiological assay for phaseolotoxin. Phytopathology *69* (1979) 663–666.

62 Staskawicz, B. J., and Panopoulos, N. J., Phaseolotoxin transport in *Escherichia coli* and *Salmonella typhimurium* via the oligopeptide permease. J. Bact. *142* (1980) 474–479.

63 Staskawicz, B. J., Panopoulos, N. J., and Hoogenraad, N. J., Phaseolotoxin-insensitive ornithine carbamoyltransferase of *Pseudomonas syringae* pv. *syringae*: basis for immunity to phaseolotoxin. J. Bact. *142* (1980) 720–723.

64 Stewart, W. W., Isolation and proof of structure of wildfire toxin (*Pseudomonas tabaci*). Nature *229* (1971) 174–178.

65 Surico, G., and DeVay, J. E., Effect of syringomycin and syringotoxin produced by *Pseudomonas syringae* pv. *syringae* on structure and function of mitochondria isolated from holcus spot resistant and susceptible maize lines. Physiol. Plant Path. *21* (1982) 39–53.

66 Taylor, P. A., Schnoes, H. K., and Durbin, R. D., Characterization of chlorosis-inducing toxins from a plant pathogenic *Pseudomonas* sp. Biochim. biophys. Acta *286* (1972) 107–117.

67 Templeton, M. D., Sullivan, P. A., and Shepherd, M. G., Phaseolotoxin-insensitive L-ornithine transcarbamoylase from *Pseudomonas syringae* pv. *phaseolicola*. Physiol. molec. Plant Path. *29* (1986) 393–403.

68 Tessi, J. L., Estudio comparativo de dos bacterios patogenos en avena y determinacion de una toxina que origina sus diferencias. Rev. Invest. Agr. *7* (1953) 131–145.

69 Thomas, M. D., and Durbin, R. D., Glutamine synthetase from *Pseudomonas syringae* pv. *tabaci*: properties and inhibition by tabtoxinine-β-lactam. J. gen. Microbiol. *131* (1985) 1061–1067.

70 Thomas, M. D., Langston-Unkefer, P. J., Uchytil, T. F., and Durbin, R. D., Inhibition of glutamine synthetase from pea by tabtoxinine-β-lactam. Plant Physiol. *71* (1983) 912–915.

71 Turner, J. G., Tabtoxin, Produced by *Pseudomonas tabaci*, decreases *Nicotiana tabacum* glutamine synthetase in vivo and causes accumulation of ammonia. Physiol. Plant Path. *19* (1981) 57–67.
72 Uchytil, T. F., and Durbin, R. D., Hydrolysis of tabtoxins by plant and bacterial enzymes. Experientia *36* (1980) 301–302.
73 Willis, D. K., Kinscherf, T. G., Coleman, R. H., and Barta, T. M., Identification and isolation of a DNA region required for tabtoxin production: apparent deletion in *Pseudomonas syringae* pv. *tabaci* variant *angula* and Tox⁻ mutants, in: Phytotoxins and Plant Pathogenesis, pp. 61–70. Eds A. Graniti, R. D. Durbin and A. Ballio. Springer-Verlag, Berlin 1989.
74 Xu, G.-W., and Gross, D. C., Evaluation of the role of syringomycin in plant pathogenesis by using Tn*5* mutants of *Pseudomonas syringae* pv. *syringae* defective in syringomycin production. Appl. envir. Microbiol. *54* (1988) 1345–1353.
75 Xu, G.-W., and Gross, D. C., Physical and functional analyses of the *syrA* and *syrB* genes involved in syringomycin production by *Pseudomonas syringae* pv. *syringae*. J. Bact. *170* (1988) 5680–5688.
76 Zhang, L., and Takemoto, J. Y., Effects of *Pseudomonas syringae* phytotoxin, syringomycin, on plasma membrane functions of *Rhodotorula pilimanae*. Phytopathology *77* (1987) 297–303.

0014-4754/91/080765-07$1.50 + 0.20/0

The genetics of phytotoxin production by plant pathogenic fungi

C. R. Bronson

Department of Plant Pathology, Iowa State University, Ames (Iowa 50011–1020, USA)

Abstract. Little is known about the genetic control of phytotoxin production by plant pathogenic fungi. The production of host-selective toxins known to play a role in disease development has been genetically analyzed in three species of *Cochliobolus*. In *C. heterostrophus*, a single genetic locus with two alleles has been identified controlling the production of HMT-toxin. This locus appears to be at or near the breakpoint of a chromosome rearrangement. Single genetic loci have also been identified controlling the production of HC-toxin by *C. carbonum* and HV-toxin by *C. victoriae*. The locus in *C. carbonum* may be a cluster of tightly linked genes.
Key words. Host-selective phytotoxins; HC-toxin; HMT-toxin; HV-toxin; *Cochliobolus heterostrophus*; *C. carbonum*; *C. victoriae*.

Introduction

Over fifty fungal metabolites have been reported to be toxic to plants and at least thirty of these are known or suspected to contribute to plant disease[18]. Nothing is known about the genes controlling the production of the vast majority of them. The purpose of this review is to introduce the reader to some of the reasons for wanting to determine the genetics of phytotoxin production and to the progress in the few systems that have been examined. Because the only genes controlling phytotoxin production identified to date are in species of *Cochliobolus*, this work will be emphasized. Prospects for progress in the genetics of phytotoxin production by *Alternaria alternata* will be mentioned briefly. There have been several previous reviews of the field[15, 25, 33, 38].

Historically, one of the prime motivations for the genetic analysis of phytotoxin production has been to test or confirm the roles of the toxins in disease development. Fungi produce numerous substances in culture that are toxic to plants[18]. Yoder[33] and others[15, 18] have argued the merits of genetic analysis as a tool for testing their significance. In essence, if the same gene or genes which control toxin production also control pathogenicity (the ability to cause disease symptoms) or virulence (the severity of the symptoms), a meaningful role of the toxin in disease development can be inferred.

The strength of the genetic test depends on the likelihood that the strains being tested differ only by their ability to produce the toxin. The most convincing tests thus far for the roles of fungal toxins in disease development have involved studies of the cosegregation of toxin production and pathogenicity or virulence in the progeny of a cross or in the progeny of a series of backcrosses. These instances will be reviewed below. Recent years have seen the development of methods for the molecular genetic manipulation of several of the phytotoxin producing fungi[25, 38]. These techniques should permit the comparison of disease induction by strains that are identical except for the precise deletion or inactivation of a structural gene controlling a terminal step in toxin biosynthesis. Modification of genes in earlier steps and regulatory genes could give suggestive information, but would be less convincing because of an increased probability of effects on other metabolic pathways.

Genetic analysis in conjunction with biochemical analysis will allow the study of how phytotoxins are made. The pathways of toxin synthesis should be inferable from mutants by observing differences in levels of intermediates in the pathway and the supplementation needed to overcome specific blocks. Such analyses would be enhanced by the cloning and functional characterization of genes involved in synthesis, regulation or secretion. The utilization of such biosynthetic information and cloned