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Bacterial phytotoxins: Mechanisms of action

R. D. Durbin

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

Abstract. Many species of phytopathogenic procaryotes produce toxins that appear to function in disease development. They affect the plant in different ways, the end result of which is the elicitation of chlorosis, necrosis, watersoaking, growth abnormalities or wilting. The most extensively studied toxins cause chlorosis. They specifically inhibit diverse enzymes, all critical to the plant cell. This inhibition results in a complex series of metabolic dysfunctions ultimately resulting in symptom expression. Substances causing growth abnormalities consist of known phytohormones and other compounds with plant hormone-like activities, but which have no structural relationship to the known hormones. The former act in the usual manner but, because of their elevated levels and imbalances, the host's regulatory mechanisms are overwhelmed and abnormal growth results (hyperplasia, shoot or root formation); the mechanisms of action of the latter group are unknown. High molecular weight, carbohydrate-containing substances, also acting in unknown ways, cause tissue watersoaking or wilting. Likewise, we know little about toxins causing necrosis except for syringomycin which affects ion transport across the plasmalemma.
Key words. Bacterial phytotoxins; tabtoxinine-β-lactam; phaseolotoxin; syringomycin; syringotoxin; rhizobitoxine; coronatine; tagetitoxin; IAA, cytokinins, ethylene; *Pseudomonas*; *Bradyrhizobium*; *Corynebacterium.*

Introduction

Members within all genera of phytopathogenic bacteria have been reported to produce phytotoxins [10]. Currently, however, only a few of these toxins have well-defined mechanisms of action. For a larger number of toxins there is physiological information as to which metabolic process(es) is disrupted and/or organelle affected, but their exact molecular targets have not yet been identified; nor, obviously, have their mechanisms of action been elucidated. For a third – and by far the largest – group of toxins, the information available on their mode of action is still too gross to make a meaningful evaluation, e.g., inhibit respiration or photosynthesis, and in many cases their structures have not been completely determined.

The coverage for this article depends, in a major way, on how one defines the term phytotoxin. Like most definitions it can either be inclusive or exclusive. I have chosen more to follow the former path and to define phytotoxins in terms of their biological interaction. Thus, molecular weight, concentration in the plant, chemical class, symptom expression, or the nature of the toxin-target interaction are not considerations for defining phytotoxins; also, enzymes are excluded from consideration [18, 54]. Phytotoxins are defined as compounds synthesized by the pathogen during pathogenesis that are deleterious to the host. Note that this definition does not exclude compounds also produced by higher plants like auxins or cytokinins. Just because at some point in time we realize that a compound which acts in the sense of a toxin also has a function in healthy plants, is not in itself ground for excluding it from consideration. What is important is that we consider only that portion contributed by the pathogen. The classical example of this situation was the discovery that *Gibberella fujikuroi* produced a mixture of gibberellins (up to 1987, 72 free gibberellins had been identified) and the demonstration that they could cause abnormal shoot elongation resembling the 'bakanae', or foolish, disease of rice seedlings incited by *G. fujikuroi*. From this work came the revelation that the gibberellins were a new class of plant growth hormones. Also included under my definition are compounds synthesized by so-called exopathogens [83].

Generally, we consider that the host's response to a toxin eventually becomes visible, e.g., chlorosis or watersoaking. However, this is not a requisite part of the definition but simply reflects the fact that this is how we originally recognized that a toxin was operative, and also probably because our initial bioassay for toxin activity was visual, i.e., we have selected for toxins that give visible evidence of their activity. It may well be that some toxins can act by changing the host's regulatory or metabolic processes in such a way as to benefit the pathogen without this deleterious effect becoming visualized, i.e., their action may only be evident at the biochemical level. Assuming this is true, we should begin to explore the use of sophisticated biochemical assays too, particularly for biotrophic interactions involving fungi. However, it may be that bacterial pathogens and their hosts have coevolved in such a way that visual evidence of phytotoxin action always results.

Because, by necessity, toxins are produced in vitro for structural characterization and mechanism of action studies, it is commonly not initially clear whether they are also produced in the plant and function as disease determinants. The symptoms produced by purified toxins 'vis à vis' the diseases are usually not very informative because the symptoms are so general and can be elicited by so many means. Occasionally though, there may be something unusual or unique about the symptom, or the way it develops, that gives one some confidence that the toxin under consideration actually does function in disease development. Attempts to show a correlation between in vitro toxin production and virulence have yielded mixed results, and, accordingly, should be viewed with a critical eye. Furthermore, the range of secondary compounds synthesized in vitro can very often be different from that synthesized in the plant, particularly with regards to different analogs[35,36]. Recovering the toxin from diseased tissues can be very difficult too because of: a) low concentration, b) binding to non-specific as well as to specific receptors, c) metabolic alterations during isolation, or d) other factors. Accordingly, only in a few instances has a toxin's presence been directly demonstrated in the plant. More commonly, the evidence for complicity rests on the observation that the toxin's in vitro target is inhibited in diseased plants, and in some cases in toxin-treated plants too. Thus knowledge of a toxin's mechanism of action requires additional biochemical and genetical evidence before one can make a persuasive argument either for or against its involvement in the plant.

With but few exceptions, bacterial phytotoxins increases disease severity, i.e., act as virulence factors – a quantitative trait[85]. In one of the exceptional cases, *Pseudomonas syringae* pv. *tabaci* isolate BR2 on bean, tabtoxinine-β-lactam production is required for disease production[26], i.e., it acts as a pathogenicity factor – a qualitative trait having to do with the ability to cause disease. Another very interesting case is that of gall formation on olive and oleander induced by *P. syringae* pv. *savastanoi*. Here, gall information is dependent upon bacterial production of indole acetic acid: IAA^- mutants do not induce galls, however, IAA overproducers induce larger galls[53]. Thus, it could be argued that IAA can function as both a pathogenicity and virulence factor depending upon the situation. It also illustrates the need for one to be flexible about definitions, for as we learn more about various systems, the more likelihood there is that the original paradigms will need modification.

Although rather commonly bacteria synthesize two or more analogs of a toxin, the active component appears to be nonvariable[36,72]. The variable portion of the analogs either presents little or no hindrance to the interaction, or it is cleaved off by hydrolytic enzymes before the toxin interacts with its target[30,34]. Other post-translational modifications are clearly possible but have not as yet been examined.

A single phytotoxin could have more than one molecular target. Here, two possibilities present themselves: a) multiple targets with the same catalytic reaction center which interacts with the toxin, or b) targets with different catalytic mechanisms but having the same or similar binding sites for the toxin. Additionally, there is no a priori reason why a toxin molecule cannot have two or more portions that can interact with different binding sites on different targets. Still another possibility is that a pathogen could simultaneously synthesize two or more toxins, each interacting with a different target. The evolutionary trend in toxins is probably towards small molecules that at low concentrations can inhibit critical points of host metabolism, the idea being to reap the maximal effect with minimal metabolic expenditure on the part of the pathogen (one might say they are highly leveraged).

The first report on the existence of a bacterial phytotoxin came from Johnson and Murwin in 1925; they showed that *P. syringae* pv. *tabaci* produced in vitro a substance that reproduced on tobacco the characteristic chlorosis caused by the pathogen[23]. Their work was extended by Clayton[7], who, besides determining some of its physical and chemical properties, showed that the toxin reduced the chlorophyll content of plant tissues. The next advance with this toxin constitutes the first truly molecular research done on how a bacterial phytotoxin acts, indeed on how any phytotoxin acts! These pioneering efforts, started in the 1940s by Braun and coworkers, generated intense interest. They proposed that the toxin, which they trivially named tabtoxin, inhibited methionine metabolism[6]. They also proposed a structure which supported their contention of how the toxin acted, i.e., it structurally resembled methionine[84]. However, later work by others showed neither to be correct[65,67,75]. Nevertheless, the line of work started by Braun's group has been the major impetus for a still-expanding series of studies on bacterial toxins and the roles they play in disease production and the pathogen's economy.

Mechanisms of action

For sake of organization, the phytotoxins to be discussed are arbitrarily arranged first under the kind of symptom they elicit and then alphabetically by toxin. As we learn more about how phytotoxins act, it should be possible to erect some logical type of classification scheme based, for example, upon their targets or type of inhibition. With such a rational system, interrelationships might become apparent and it could have predictive value, too. This synopsis updates earlier reviews [11, 19, 35, 44, 48].

Chlorosis

Coronatine [22]. This toxin is synthesized by several pathovars of *P. syringae*, including *atropurpurea*, *tomato*, *glycinea*, *maculicola* and *morsprunorum*. These pathogens cause chlorotic halo blights in which coronatine has been hypothesized to cause the chlorotic response; at elevated concentrations coronatine also can cause a necrotic reaction in the central portion of the spot [43]. Some isolates of pv. *tomato* produce a different toxin which causes a faint chlorosis [39] and pv. *glycinea* produces a toxin acting much like phaseolotoxin [16]. Their identity and significance in pathogenesis remains to be characterized. Several groups have shown that coronatine production is plasmid controlled, but whether in all cases the plasmid DNAs are identical and contain all the structural genes for toxin synthesis has not been determined. However, initial experiments point to at least major homology between the plasmids contained by the pathovars *tomato* and *atropurpurea* [63].

Current mechanism of action studies are focused on the ability of coronatine to cause hypertrophy of potato tuber discs and open stomata of Italian ryegrass. In the former case, water uptake has been postulated to depend upon: a) an increase in cell wall plasticity which requires aerobic respiration, protein synthesis and increased cell wall degrading enzyme activities [59, 61], b) an alteration in the plasmalemma, possibly related to ATPase activity [57, 58], and/or c) an increase in osmotic pressure brought about by starch hydrolysis [60]. Presumably, some of these factors could also be involved in the ability of coronatine to cause stomatal opening [33] but what, if anything, they have to do with chlorosis is unclear.

The results suggest that coronatine may act much like a plant growth hormone, especially IAA [62]. Additionally, Mitchell and coworkers [35] have shown that coronatine stimulates ethylene production. Also when they added aminoethoxyvinylglycine (AVG), a known inhibitor of ethylene biosynthesis, this stimulation was inhibited. However, auxin can induce AVG-sensitive ethylene biosynthesis [86]. Thus, at this point it is not clear whether coronatine's primary action is related to either of these phytohormone activities, or even whether it might have several sites of action.

Phaseolotoxin [40]. Patel and Walker [47] were the first to call attention to the marked accumulation of ornithine in bean tissues infected by *P. syringae* pv. *phaseolicola*, and they suggested that the toxin might affect amino acid metabolism. Semi-purified toxin preparations can cause a similar accumulation of ornithine [56]. Both arginine and citrulline, applied singly 48 h after the toxin, can partially reverse chlorosis [50] and citrulline can completely protect against chlorosis if administered at or prior to toxin treatment; arginine protection was not tried [49]. Patil and coworkers [49] showed that phaseolotoxin specifically inhibited an enzyme in the ornithine cycle, ornithine carbamoyltransferase (OCTase). They concluded, using Lineweaver-Burk plots, that the toxin was noncompetitive with respect to ornithine but competitive with respect to carbamoylphosphate [70]. They interpreted these results to mean that phaseolotoxin binds to an allosteric site distinct from the site occupied by carbamoylphosphate. Binding, by inducing some type of a conformational change in the enzyme, resulted in a decreased affinity for carbamoylphosphate. In further work they suggested that phaseolotoxin was an active site directed irreversible inhibitor [29]. The biological relevance of these works has been called into question by studies of Templeton and coworkers [73], who found that in the plant phaseolotoxin is converted to N^{δ}-(N^{1}-sulpho-diaminophosphinyl) ornithine (PSorn) by hydrolysis, and that it is this compound that interacts with OCTase. They reported that PSorn is a potent, second-order inactivator. Carbamoylphosphate, but not ornithine inhibits this inactivation, suggesting that PSorn is acting at the active site of OCTase. Inhibition of OCTase can be linked to chlorosis by several means: a reduction in protein synthesis because of an arginine deficiency [50, 70], or a depression in pyrimidine synthesis because of a citrulline deficiency which in turn would reduce carbamoylasparate levels [54]. The results from recent investigations have suggested that chlorosis is due to a reduction in chlorophyll biosynthesis brought on by a deficiency of arginine [79].

Rhizobitoxine [25]. Originally isolated from strains of *Bradyrhizobium* (*Rhizobium*) *japonicum* causing apical chlorosis of soybean, rhizobitoxine also recently has been identified as a product of *Pseudomonas andropogonis*, cause of maize stripe [36]. The toxin has been shown to react with two different pyridoxal phosphate-linked enzymes. The first, β-cystathionase, is critical for sulfur-containing amino acid biosynthesis [45]. Additional kinetic studies using spinach β-cystathionase are consistent with the notion that rhizobitoxine is an active site directed irreversible inhibitor [15]. Rhizobitoxine also was found to inhibit ethylene production [46] not, it was postulated, by inhibiting methionine synthesis but rather by directly blocking the conversion of methionine to ethylene. This led directly to the suggestion that a pyridoxal phosphate-linked enzyme reaction might be involved in ethylene biosynthesis. Somewhat later, it was found that the toxin indeed inhibited the conversion of *S*-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid (ACC), an intermediate in the ethylene biosynthetic pathway [1]. The

enzyme responsible for this conversion, ACC synthetase, as had been postulated, is pyridoxal dependent. Whether the inhibition of one or both of these enzymes, or perhaps some other enzyme, is linked to chlorosis has as yet not been examined. Since chlorosis is essentially confined to the new leaves where chloroplast development is rapidly taking place, it may be that chlorosis is a result of a methionine deficiency brought about by β-cystathionase inhibition.

Tabtoxinine-β-lactam[3,67,72]. This toxin is produced by a series of *P. syringae* pathovars that induce chlorotic halos surrounding necrotic centers[77]. Generally, the pathogen cannot be isolated from chlorotic areas but is restricted to the necrotic zones. In tobacco, in particular, the toxin may become systemic and cause a generalized chlorosis of a large part of the canopy. Depending upon the strain of the pathogen, it may produce only the dipeptide, protoxin form, trivially named tabtoxin, which then is enzymatically hydrolyzed to tabtoxinine-β-lactam when it enters the host cell[80]. More usually though, the bacterial strains have in their periplasm a Zn-containing amino peptidase which hydrolyzes the protoxin[30]. At one time tabtoxin was reported to inhibit 'Rubisco'[8], but more recent work has refuted this claim[76]. As far as we know now, tabtoxin has no biological activity, and it is tabtoxinine-β-lactam that is the biologically active form of the toxin. The tabtoxinine-β-lactam moiety chemically isomerizes to its more stable δ-lactam form, especially at basic pHs, but these products (isotabtoxin and tabtoxinine-δ-lactam) do not have biological activity either[71]. Several early findings are significant for our understanding of the toxin's mode of action: a) light is required for chlorosis development, b) ammonia accumulates as chlorosis develops, c) glutamine can protect against chlorosis and d) methionine sulfoximine (MSO) inhibits glutamine synthetase (GS). These findings were of great help in leading us to show that tabtoxinine-β-lactam is an irreversible inhibitor of GS[75]. The inhibition leads to a rapid buildup of ammonia in the light since GS is involved in the photorespiratory recycling of ammonia released during the conversion of glycine to serine[14,78]. Probably, it is this ammonia that is most important in causing the initial chlorosis by rapidly disrupting the chloroplast's internal membrane system[9]. However, the blockage of synthetic processes in which GS is involved and the uncoupling of photosynthesis, assuming the ammonia levels are sufficiently high in the chloroplast, may be important later on and cannot be discounted at this time[77].

Tagetitoxin[38]. This toxin is produced by *P. syringae* pv. *tagetis*[37]. The chlorosis it causes is very characteristic in that it is almost exclusively confined to the host's apical meristems; occasionally though, chlorotic halos will develop around the primary necrotic lesions on the already formed, lower leaves where the bacterium is found. The apical occurrence results from the translocation of the toxin from the primary lesions to the apices and because the metabolic state of developing chloroplasts is particularly sensitive to the toxin.

Ultrastructurally, the chloroplast appears to be the only affected organelle[24]. Of particular importance is the fact that in chlorotic cells there is an almost complete absence of chloroplastic 70S ribosomes, whereas these cells still have an essentially normal complement of cytoplasmic 80S ribosomes[31]. Northern analysis using probes specific for chloroplast 16S and 23S or cytoplasmic 18S and 25S rRNA subunits have shown that the toxin's effect is specific to chloroplast ribosomes. These results further confirm that the toxin preferentially affects the chloroplast.

Western blots and silver-stained gels have shown that the toxin has relatively little overall effect on the soluble proteins of the cell except for 'Rubisco', which is essentially absent. Tagetitoxin has no effect, in short-term experiments, on the rate of protein synthesis in isolated intact chloroplasts; it only slightly inhibits DNA synthesis, probably because of its requirement for RNA; but it immediately and strongly inhibits RNA formation. Tagetitoxin also very significantly reduces the RNAs for the large subunit of 'Rubisco', the 32 kDa quinone-binding thylakoid protein and the β and ε subunits of chloroplast coupling factor 1, all of which are encoded by chloroplast genes.

These and other results have led to the conclusion that chloroplast RNA polymerase is the site of tagetitoxin action[32]. Although the exact nature of this interaction is yet to be resolved, we do know that tagetitoxin does not interact with the DNA template alone, or inhibit substrate binding, open complex formation or phosphodiester bond formation. The current hypothesis is that it directly interacts with the RNA polymerase, inhibiting some step(s) closely related to translocation and/or product release.

Growth abnormalities

It is beyond the scope of this presentation to describe what is known about the mechanisms of action of phytohormones; indeed, even after many years of effort we still do not know in any case exactly how their action is exerted. Suffice to say, a good deal of effort is currently being placed on hormone receptors and their relationship to the physiological effects induced by the hormones[82].

Cytokinins[68]. *Corynebacterium fascians* induces a fasciation or 'witches broom' of pea which is characterized by a release from apical dominance and growth of the lateral buds. In culture the bacterium produces at least nine N^6-isoprenoid adenine analogs, the most abundant being N^6(Δ^2-isopentenyl) adenine (i^6Ade), which is probably the most widely distributed cytokinin in plants too[27]. Since, like the disease, these cytokinins produce fasciation in plants, it has been proposed that this disease symptom is due to cytokinin production by the pathogen[74]. This is an attractive idea but as yet there is no

definitive confirmation that such is the case [52]. Nevertheless, most of the evidence points towards this conclusion. Isolates of *P. syringae* pv. *savastanoi*, causal agent of olive knot, also produces a broad diversity of cytokinins, ranging from i^6Ade, its ribosyl derivative, i^6A, *cis*-zeatin (c-io^{6}Ade), 1″-methylated zeatins to the novel cytokinin, 1″-methyl-*trans*-zeatin riboside [53]. Their biosynthesis is controlled by genes contained on a 41 kb plasmid. Genetic evidence has shown that the bacterium's ability to induce hyperplasia is positively and obligatorily related to its ability to produce cytokinins [16]. Zeatin (t-io^{6}Ade) and i^6Ade, plus two other unidentified cytokinins, were detected in the culture filtrates of *P. amygdali*, the recently discovered cause of almond canker [21]. Significantly larger amounts of t-io^{6}Ade were produced by highly virulent strains as opposed to weakly virulent ones. Since the pathogen causes a cortical hyperplasia which slowly splits the bark, the authors suggested that these plant growth substances are likely involved in symptom development. This same group later identified one of the two unknowns, a new naturally occurring 2′-deoxyzeatin riboside [13a].

Ethylene [68]. Although the increased production of ethylene is probably a ubiquitous result of infection, very few pathogens have been shown to synthesize it. For those that do, one is immediately faced with the problem of how to interpret in vitro ethylene production in terms of the in-plant situation. Are the two conditions comparable and how does one determine the contribution of the pathogen to the elevated ethylene levels present in the diseased plant? A similar dilemma exists for IAA [52]. The critical application of site-specific mutants could be very helpful for clarifying these controversies.

Indole acetic acid [68]. This plant growth hormone is synthesized by a variety of pathogenic bacteria; however only in the case of *P. syringae* pv. *savastanoi* has its involvement in disease production been thoroughly established. Strains of the pathogen causing galls on oleander contain the genes encoding IAA synthesis on a plasmid, pIAA, which is distinct from the plasmid bearing the cytokinin genes. In strains attacking olive, these IAA-synthesizing genes are on the chromosome [53]. As indicated earlier, there is complete correspondence between the ability to produce galls and the presence of a functional IAA synthesizing operon [41]. In nature, IAA and the cytokinins discussed above act in concert to produce the hypertrophic and hyperplastic overgrowths at the infection sites [13, 28]. Possibly, almond canker represents a similar case, as *P. amygdali* produces IAA and its methyl ester as well as the cytokinins mentioned earlier [21].

Necrosis

Syringomycin [64]. This toxin is produced by many strains of *P. syringae* pv. *syringae*, causing among other diseases, brown spot of bean, holcus spot of maize and many other Gramineae, and bacterial canker of stone fruits [20]. It was first reported in the late 1960s; however, it was not until 1989 that the complete structure was elucidated. These results showed that the toxin is, in reality, composed of a mixture of related lipodepsinonapeptides rather than a single molecular species. Presumably, all the analogs (the three reported ones vary in their fatty acid moieties) have the same site of action. However, it's possible that some may vary in toxicity or not be toxic per se but rather modulate the toxicity of the other analogs. In this regard we need to remember that all but the most recent mechanism of action studies have been done with impure preparations.

Results, first from DeVay's group and more recently from Takemoto's, have shown that syringomycin affects plasmalemmial ion transport processes rather than causing a general effect on membrane integrity, as was first thought [2, 69]. An antibody specific against the toxin has been observed to accumulate around cell surfaces in the phloem, xylem, cambium and pith of peach, supporting the idea that syringomycin is produced in infected plants where it interacts with the plasma membrane [51, 66].

Bidwai and coworkers [4] have suggested that the toxin acts by stimulating a membrane-bound protein kinase which phosphorylates several membrane polypeptides, among them the plasmalemmial, but not mitochondrial or tonoplast, H^+-ATPase [5]. Zhang and Takemoto [87, 88] showed that syringomycin causes membrane hyperpolarization and alters proton and potassium fluxes, suggesting that syringomycin stimulates a H^+/K^+ antiport. This alteration in electric potential and H^+ gradient across the plasmalemma maybe at the root of syringomycin's action. Further work has shown that the toxin-induced phosphorylation is related to transient Ca^{2+} fluxes across the membrane [69]. Possibly, Ca^{2+} is required for protein kinase activity, although other scenarios are also possible.

Interestingly, the toxin closely mimics abscisic acid (ABA) by inducing stomatal closure [42]. The two compounds act at similar rates at similar concentration (as low as 10^{-8} M on epidermal strips). Closure in both cases is Ca^{2+} dependent and is reversed by fusicoccin. Although the two toxins may differ in how they initially affect the guard cell, both appear to activate a pump promoting K^+ efflux. These findings are consistent with the proposed H^+/K^+ antiport system. It appears that if the physiological and biochemical actions of these two compounds continues to coincide, further studies on syringomycin may well tell us much about ABA action too.

Syringotoxin [17]. Produced by strains of *P. syringae* pv. *syringae* causing blast of *Citrus* spp., this toxin behaves biologically like syringomycin but differs in chemical composition. How it acts is unknown.

Watersoaking

Extracellular polysaccharides [55]. Rudolph's group has for some years been working on the role of bacterial extracellular polysaccharides (EPS) in susceptible and resistant reactions. The chemistry of this group is poorly under-

stood because it consists of a mixture of high molecular weight, structurally complicated substances. However, some progress has been made, especially with *P. syringae* pv. *phaseolicola*. Its main EPS components consist of a mannuronan (alginate) and a fructan (levan). There is a high correlation between EPS production, especially the mannuronan, and virulence, EPS is produced in infected plants and by itself can cause watersoaking, and EPS$^-$ mutants do not cause watersoaking. However, how EPS acts is still unknown.

Wilting

This topic has recently been reviewed by Van Alfen[81]. Toxins in this group act primarily by changing the extracellular water potential, affecting either stomatal function or increasing water flow resistance. Other mechanisms, especially a direct effect of the toxins on membrane function, have been suggested but there is little evidence at this time to support them.

Discussion

Determining a toxin's mechanism of action is certainly significant, but it still provides only a partial answer to the question: what is the toxin's role in pathogenesis? How centrally important is it? We need to know a lot more about the toxin: what is its localized concentration in various tissues and subcellular compartments; is its production regulated and if so how; what metabolic conversions does it undergo in the plant; does it interact with other pathogenic determinant(s); what is the nature of the target molecule and its binding site; is the target a direct effector or is it coupled to effectors via some sort of signal transduction mechanism; what transcriptional, metabolic and/or structural connections exist between the primary metabolic lesion and the subsequent expression of symptoms and ultimately yield loss; and finally, how are all these factors modulated during disease development? Hopefully, once we have such information in hand, we can rationally – rather than empirically – develop strategies to obviate the effects of toxins on plants. The applications range from screening for disease resistance via cell or tissue culture selection to the design of molecules that can antagonize toxins, the use of toxins in weed control, and the molecular manipulation of self-protection mechanisms[12].

Aside from these considerations, there is a broader question: what role or roles do toxins play in the bacterium's life history? Does understanding their action in infected plants describe their sole function or is there perhaps some other 'raison d'être'? Answering these questions will require a thorough understanding of toxin action. Clearly, much progress has been made in this regard but much still remains to be done.

Note added in proof. Tolaasin, produced by *Pseudomonas tolaasii*, causes pitting and browning of mushroom caps. It occurs as two unusual lipodepsipeptides varying at residue 17 (Hse or Gly). The molecule forms a left-handed α helix having amphiphilic character perhaps important for its membrane-active properties (Nutkins et al., J. Am. chem. Soc *113*: 2621–2627). Tolaasin has voltage-dependent ion channel activity which causes cell lysis (Rainey et al., Adv. Plant Path., in press). The structures of syringomycin E (Fukuchi et al., Tetrahedron Lett. *31*: 1589–1592) and syringotoxin (Ballio et al., FEBS Lett. *269*: 377–380) have been eluciated. The syringomycins now seem to be mainly responsible for the antimicrobial activity of *P. syringae* pv. *syringae*, whereas the syringopeptins, a newly identified, more hydrophobic subgroup of compounds related to the syringomycins, are responsible for phytotoxicity (Iacobellis et al., Physiol. molec. Plant Path., in press). Another subgroup of lipodepsipeptides, the syringostatins, has also been isolated from *P. s.* pv. *syringae*., (Isogai et al., Tetrahedron Lett. *31*: 695–698). Their mode of action is unknown but probably is similar to that of the syringomycins.

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Non-host-selective fungal phytotoxins: Biochemical aspects of their mode of action

A. Ballio

Dipartimento di Scienze Biochimiche, Università 'La Sapienza', I-00185 Roma (Italy)

Abstract. During the last decade increasing attention has been directed towards the biochemical mechanisms responsible for the biological activity of phytotoxins. Studies on the mode of action of some non-host-selective phytotoxins, some following on from previous observations, have demonstrated a very specific interaction with particular components of the cell machinery, and have suggested the possible use of these phytotoxins as tools for the investigation of important biochemical processes. This review article reports and discusses results of studies carried out in the 1980s with seven non-host-selective fungal toxins: brefeldin A, cercosporin, *Cercospora beticola* toxin, fusicoccin, ophiobolins, tentoxin, and zinniol. Each of these interferes with the life of the host by interacting with a different biochemical target.

Key words. Non-host-selective phytotoxins; brefeldin A; cercosporin; *Cercospora beticola* toxin; fusicoccin; ophiobolins; tentoxin; zinniol; receptors; plasma membrane; endoplasmic reticulum; ATPase; calmodulin; oxygen radicals.

Introduction

Until 1980, when the state of knowledge of the mode of action of phytotoxins was reviewed by Daly [32], very little work had been devoted to understanding the molecular mechanisms responsible for the toxicity of these plant metabolites. In recent years, interest in this aspect of phytotoxin research has increased, and it is to be hoped that the results will stimulate future investigations.

So far, more attention has been directed towards host-selective than non-host-selective phytotoxins; the obvious involvement of the former in disease makes them more attractive for biochemical and genetic investigations. On the other hand, non-host-specific phytotoxins often act